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SUMARIO

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FE DE ERRATA

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ELUTION DIAGRAMS OF NUCLEOTIDES FROM LEUKEMIC AND NORMAL BLOOD

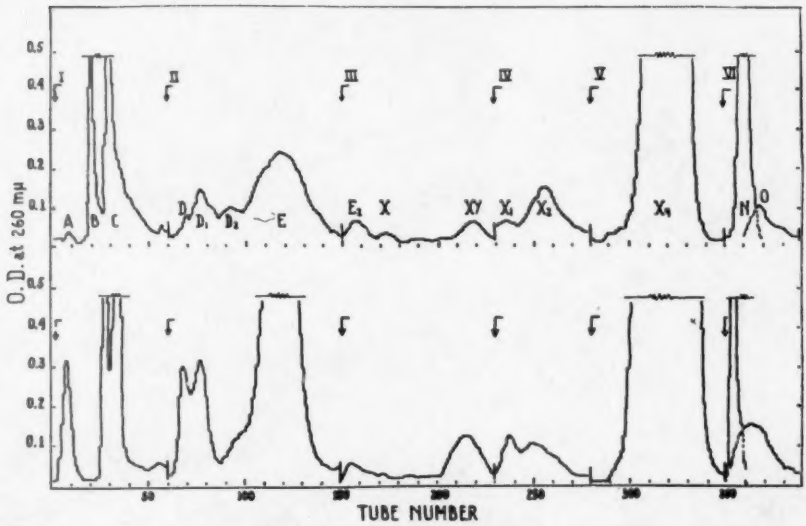
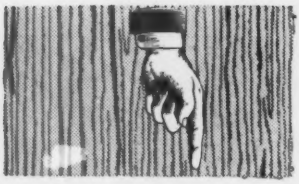


FIG. 1. — *The elution diagram corresponds to λ 260 mμ absorbing material separated with columns of Dowex 1 Cl' x 8 of 1.2 x 20 cm. The eluates were collected in a LKB automatic fraction collector in volumes of 5 ml/tube. The solvents utilised were: I. 0.005 N HCl; II. 0.01 N HCl — 0.01 M NaCl; III. 0.01 N HCl — 0.03 M NaCl; IV. 0.01 N HCl — 0.05 M NaCl; V. 0.01 N HCl — 0.10 M NaCl; and VI. 0.01 N HCl — 0.50 M NaCl. The upper part of the figure corresponds to the perchloric acid soluble nucleotides from a 40 ml sample of leukemic blood (see L2, table I and II). The lower part of this figure represents the nucleotides from a 97ml sample of normal blood (see N5, table I and II).*



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ÓRGANO OFICIAL DE LA
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EFFECT OF STORAGE ON IODINE 131 SOLUTIONS (*)

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DOCTOR and Trunnell (1), Doctor (2) and Taurog, Potter and Chaikoff (3) found several extraneous bands in radioautographs of chromatograms of stored Na I^{131} solutions from Oak Ridge. Doctor and Trunnell systematically studied during one month the chromatographic behaviour of Na I^{131} solutions and showed that at the end of four weeks only 16 % of the total radioactivity was due to Iodine. Thirty two per cent of the total radioactivity was due to a band, which upon elution and injection into one day old chicks, showed a thyroid uptake of only 20 % as compared with 60 % shown by the Iodine. Besides this extraneous band, chromatograms showed other bands which did not belong to Iodine. The present study was undertaken to investigate whether the impurities observed by Doctor and Trunnell also appear in the Na I^{131} used in Argentina for clinical and biological research. This Iodide comes almost entirely from the Radiochemical Center of Amersham, England.

(*) This work has been partially supported by a grant of the "Consejo Nacional de Investigaciones Científicas y Técnicas" of Argentina.

Received for publication, December 9, 1960.

MATERIAL AND METHODS

Three batches of Amersham, carrier free Na I^{131} in dilute sodium thiosulfate have been used. Approximately 0.5 μC of Na I^{131} were deposited at the origin of strips 30 cm long and 3 cm wide. The radioiodine was diluted so that four micropipets contained the required amount. Chromatograms were run fourfold during 20 hours, one-dimensionally, in an ascending technique and parallel to a strip of 0.5 mg of Na I^{127}. A doublefold run of 4 μC of Na I^{131}, also contained in 4 micropipets, was carried out. Fourfold runs of 0.5 μC were repeated every 8 days. n-Butanol equilibrated overnight with 3N ammonia solution as solvent system was used. Before the runs, the cube was equilibrated for a minimun of 5 hours. Whatman Nº 1 paper was used for chromatography and Kodak blue band film for radioautography. Of each run, films were exposed to two strips during 48 hours and to two other strips during 4 days.

Optical density of the I^{131} band and of the band at the origin was determined on typical strips. A linear scale densitometer was used, having a precision of 1/10 000 of maximum trans-

mittance. It was intended by means of densitometry to establish unimodality of Na I^{131} bands as well as similarity in their patterns.

Results and Discussion

1) In all chromatograms a sharp band with an rF equal to 0.3 was obtained, parallel to the one shown by Na I^{127} upon inmersion in silver nitrate solution. This rF corresponds to iodide anion in similar systems (4).

2) For all batches, narrow single bands at the origin were obtained at some moment. Small variations between batches in relation to week of appearance of said band and time of exposure necessary to show up were observed. The intensity of these bands, as shown by their optical density, was practically insignificant in relation to the intensity of I^{131} bands (Fig. 1).

3) In order to establish whether the band at the origin belonged to a preexisting element of longer half life than I^{131} or to an element which appears during desintegration of I^{131}, a solution of 4 μC, 8 times as concentrated as the previous, corresponding to the first week was chromatographed. In this experience, the band at the origin appears right from the beginning.

4) Wider bands of I^{131} (1.5 to 2 cm) than those obtained by Doctor and Trunnell, were observed. Photometric measuremets of these bands showed, however, unimodal curves which are similar in their patterns. Bands can thus be considered as single and homogeneous, in spite of their bigger extension, and not as two or more overlapping bands.

The results obtained show that racioactive Iodine from Amersham, does not present the inconveniencies found

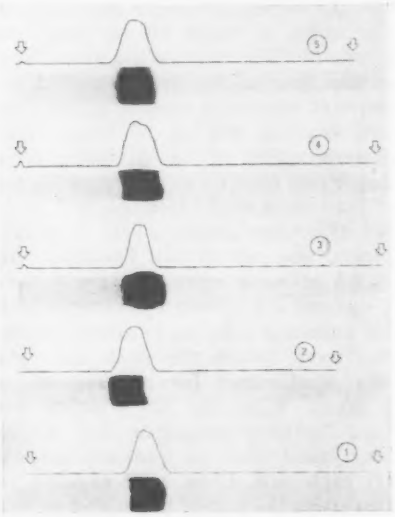

FIG. 1. — *Chromatogram of 0.5 μC of Na I^{131} stored for 1, 2, 3, 4 and 5 weeks. Arrows indicate origin and solvent front. Very weak bands at the origin on the radioautographs, as those belonging to strips 4 and 5, do not appear in the photographic reproduction.*

TABLE I

Chromatography of Na I^{131} during 5 weeks: appearance of bands at the origin

Batch	Weeks									
	1		2		3		4		5	
	a	b	a	b	a	b	a	b	a	b
72381	—	—	—	—	+	+	+	+	+	+
69191	—	—	—	+	+	+	+	+	+	+
70310	—	—	—	+	+	+	+	+	+	+

a and *b*: exposures for 2 and 4 days.
—: no bands at the origin.
+: appearance of bands at the origin.

by Doctor et al. in Oak Ridge Iodine ([1, 2]). Nevertheless, we think it usefull to analyze I^{131} solutions by chromatography routinely and on a periodic basis.

Summary

The existence of bands extraneous to I^{131} was investigated. For this purpose, three solutions of Na I^{131} from the Radiochemical Center, Amersham, England, systematically chromatographed during 5 weeks, were used. A narrow band at the origin is the only evidence of impurity. This band shows variable intensity but is not important enough to justify variations in Iodine uptake by thyroid or other tissues. As different results were obtained by other workers with radioiodines of different origin, a routine and periodical autoradiographic analysis of I^{131} solution used in endocrinology is advisable.

Acknowledgements

Thanks are given to J. R. Cordero Funes for his valuable advice and discussion, to M. del C. Armelín for technical assistance and to the "Comisión Nacional de Energía Atómica" of Argentina for the provision of Na I^{131}.

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INTERRELATIONSHIPS BETWEEN SERUM CREATININE, UREA, SULFATE AND ENDOGENOUS CREATININE CLEARANCE IN MAN

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IN a previous publication (1) we have studied the relationship between serum creatinine level and endogenous creatinine clearance by means of empirical formulae. This approach is useful in describing the phenomenon independent of the mechanism by which creatinine is excreted. The present publication deals with further studies using the same approach but including serum urea and sulfate levels as a function of the endogenous creatinine clearance.

MATERIAL AND METHODS

The same human material used in the previous publication (1) has been used for this study, the serum being kept in the frozen state after the determination of the creatinine clearance. For the present study, the serum was warmed up to room temperature and after vigorous shaking was analyzed for urea by the Conway (2) technique and for inorganic sulfate by the technique of Power and Wakefield (3). Table I summarizes the results and includes, in addition to the endogenous creatinine clearance and serum creatinine figures used in the previous study, the figures of urea and inorganic sulfate determined in the present work.

ANALYSIS OF DATA

The following symbols are used:

C: endogenous creatinine clearance in cc/min.
Crs: serum creatinine concentration in mg/100 cc.
Us: serum urea concentration in mg/100 cc.
Ss: serum inorganic sulfate concentration in mEq/100 cc.
SD: standard deviation
r: coefficient of linear correlation
log: decimal logarithm

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TABLE I

Case Nº	Clearance Period 1	2	Average	Serum Creat.	Urea	SO_4 mEq/L
	cm^3/min			mg/100 ml		
1	1	1	1	27.4	485	10.36
2	3	3	3	18.5	320	4.77
3	3	3	3	23.5	447	9.68
4	3	3	3	7.0	298	4.85
5	3		3	15.0	309	
6	4	4	4	26.3	370	9.45
7	4	6	5	10.3	150	3.31
8	6		6	6.5	171	3.15
9	9	9	9	8.4	181	
10	9	9	9	6.2	208	
11	10		10	8.6	123	2.58
12	13	13	13	8.1	143	2.68
13	15	14	14	4.5	109	1.92
14	14	16	15	5.9	65	
15	21	21	21	3.2	82	
16	25	18	21	2.8	85	1.71
17	30		30	2.6	103	1.25
18	37	31	34	3.1	39	
19	30	38	34	3.1	38	1.10
20	41	44	42	1.1	53	1.19
21	51	48	49	1.7	62	1.58
22	58	54	56	0.8	23	1.48
23	58	57	57	1.4	56	
24	74	69	71	1.1	71	
25	68	75	72	1.1	23	1.19
26	79	74	76	1.4	44	0.91
27	77	77	77	1.4	57	
28	83	75	79	1.7	41	
29	93	81	87	1.1	27	
30	94	116	105	1.1	29	1.53
31	105		105	1.2	41	
32	107	108	107	0.8	47	0.69
33	109	113	111	1.5	41	0.88
34	114	113	113	1.4	63	0.99
35	110	118	114	1.2	57	
36	127	128	127	0.8	22	

Creat., creatinine.

The regression equations have been calculated by the method of least squares. The prediction errors have been calculated by taking the standard deviations of the regression equations as a percentage of the dependent variable. This method of calculation is illustrated by Effersøe (4). The relationship between the serum variables has been studied by means of linear regression equations and by means of hyperbolic equations derived from the relationship between the serum variables and the endogenous creatinine clearance.

Results

The serum concentration of creatinine, urea and inorganic sulfate as functions of the endogenous creatinine clearance:

The mathematical relationships are as follows (see figures 1, 2 and 3):

$Crs = 10^{1.573}\ C^{-0.769}$; SD = 0.149; Prediction error + 41 %, − 29 %
$Us = 10^{2.735}\ C^{-0.586}$; SD = 0.201; Prediction error + 59 %, − 37 %
$Ss = 10^{1.013}\ C^{-0.534}$; SD = 0.130; Prediction error + 35 %, − 26 %

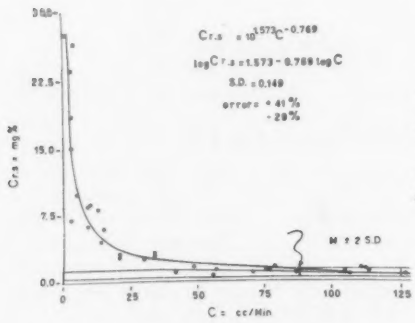

Fig. 1.

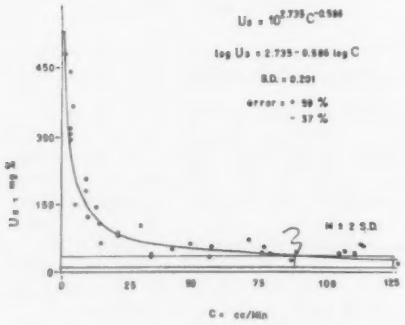

Fig. 2.

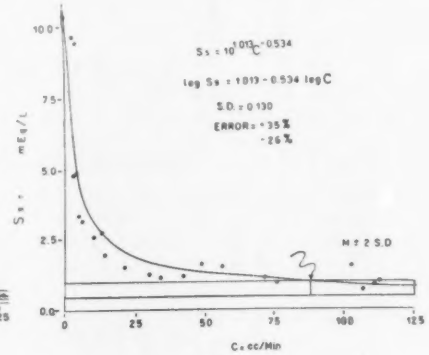

Fig. 3.

It may be seen that the equations are of the hyperbolic type. SD refers to the standard deviation of the regression equations and it allows to calculate the prediction errors. This calculation is detailed in Effersøe's paper (4). The largest prediction errors correspond to the equation for urea and the smallest, to the equation for sulfates.

The endogenous creatinine clearance as a function of the serum concentration of creatinine, urea and inorganic sulfate:

The mathematical relationships are as follows:

$C = 10^{1.984}\ Crs^{-1.181}$; SD = 0.185; Prediction error + 53 %, — 38 %
$C = 10^{4.164}\ Us^{-1.440}$; SD = 0.240; Prediction error + 74 %, — 42 %
$C = 10^{1.817}\ SS^{-1.635}$; SD = 0.228; Prediction error + 69 %, — 44 %

It may be seen that the prediction errors are larger when the clearance is calculated from the serum figures. Again, the equation for urea gives the largest error. In this type of prediction, the equation for creatinine gives a smaller error than the one for sulfate.

Interrelationship between serum concentration of creatinine, urea and inorganic sulfate:

Figures 4, 5 and 6 contain this information. As the coefficients of linear correlation were high, straight line regression equations were used to fit the data and, as may be seen, they do so adequately. Additional equations were obtained from those correlating serum variables with clearances (Fig. 1, 2 and 3). An example illustrates this point.

From the previously calculated equations (Fig. 1 and 2):

$Crs = 10^{1.573}\ C^{-0.769}$
$Us = 10^{2.735}\ C^{-0.586}$

we may derive the following equation relating urea and creatinine:

$Us = 10^{1.536}\ Crs^{-0.762}$

This equation is of the hyperbolic

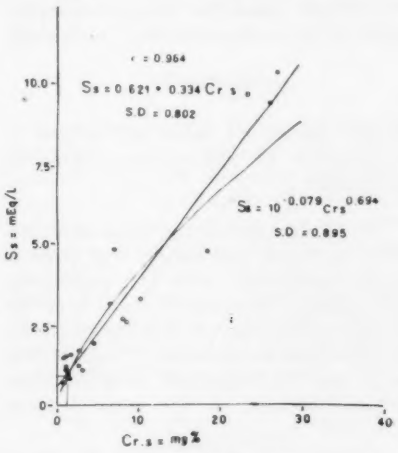

FIG. 5.

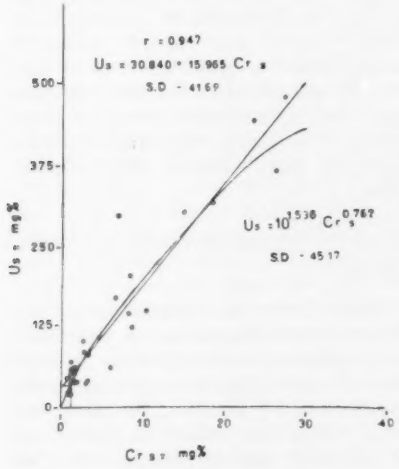

FIG. 4.

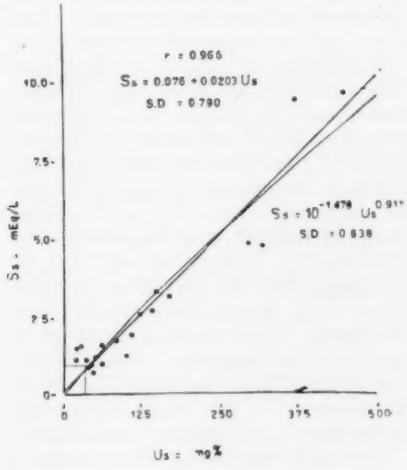

FIG. 6.

type and corresponds to the curvilinear regression line of Fig. 4. The same method of calculation has been used to draw the curvilinear regression lines of Figures 5 and 6. For this type of derivation, equations have been selected with the clearance as an independent variable, because serum concentrations depend on renal function, but renal function is not physiologically dependent upon the serum concentrations of the substances here considered.

Discussion

Serum creatinine, urea and sulfate as a function of endogenous creatinine clearance:

As in the case of the creatinine studies reported previously, the present study shows that there is a hyperbolic relationship between serum urea or sulfate concentration and the clearance of endogenous creatinine. This relationship may be expressed as a mathematical function of the general form $Y = AX^{-k}$.

Figures 1, 2 and 3 show an arrow pointing to the intersection of the minimum endogenous creatinine clearance and the maximum normal serum concentration of creatinine, urea and sulfate respectively. This point has been determined by taking two standard deviations below the mean for the endogenous creatinine clearance and two standard deviations above the mean for the serum concentration in a large group of normal men studied in this laboratory (5). It may be seen that in the three cases the regression line deviates from the normal figures near this point. This means that there is not a marked reduction of renal function before the serum figures rise above the normal, as has been interpreted by McKay (6), Van Slyke (7) and others.

Effersøe (4) has shown that in patients with renal insufficiency, but in a steady state of serum creatinine concentration, it is possible to predict the endogenous creatinine clearance from the serum creatinine concentration with an approximate error of 20 %. We have previously shown that in our group of unselected patients this error is + 47 %, — 32 %. The present report shows that the clearance is predicted from the serum urea figures with an error of + 59 %, — 39 %; using the serum sulfate figures the error is of + 35 %, — 26 %. The errors are much too large to permit the prediction of clearance knowing only the serum levels in this group of unselected cases.

The finding of larger prediction errors when the clearance is calculated from the serum levels than the one found for the inverse prediction, can be explained physiologically because the serum concentrations depend on renal function but the inverse dependence is not necessarily true. It is interesting to observe that the smallest prediction error corresponds to the equation where the serum sulfate concentration is a function of the clearance. If we assume that man has a small sulfate Tm, as has been demonstrated in dogs by Lotspeich (8), patients with renal failure and sulfate retention should show a good correlation between serum concentration and filtration rate. Our data would favor this hypothesis if we accept that the creatinine clearance approximates the filtration rate.

Interrelationships between the serum variables:

When urea was studied as a function of creatinine, sulfate as a function of creatinine and sulfate as a function of urea (Figures 4, 5 and 6) the coefficients of linear correlation between these concentrations were high in all cases and the linear regression equations described the facts adequa-

tely. From the functional relationship between the serum variables and the endogenous creatinine clearance, equations were derived which correlated the serum variables among themselves. This relationship proved hyperbolic, but the regression lines are almost linear in the experimental range. Figures 4, 5 and 6 show that the hyperbolic curves fit the data adequately. The standard deviations, although somewhat large, are very close to the ones corresponding to the straight line regression. These observations, we believe, offer a good check on the validity of the empirical equations selected to describe the relationships between the serum variables and the clearances.

The equations which contain the serum values as a function of the clearance (Fig. 1, 2 and 3), allow us to appreciate the rate of increase in serum levels. Being equations of the log-log type, a given percentage change in the clearance produces a constant percentage change in the serum values. The equations of figures 1, 2 and 3 show that in terms of percentage, the rate of increase in concentration in the blood is fastest for creatinine (largest exponent) and smallest for sulfate (smallest exponent). The difference between urea and sulfate is small.

Hamburger and Richet [9] and Masson, Crosnier and Richet [10] have recently emphasized the proportionality which exists between the concentrations of urea and inorganic sulfate in the blood. Figure 6 shows this very clearly. The straight regression line passes through zero (simple proportionality) and the hyperbolic equation $Ss = 10^{-1.478}\ Us^{0.911}$ shows an exponent close to unity. An exponent of one would convert this equation into a simple proportionality. From the clinical point of view, this relationship is important because it indicates that a high blood urea level is an expression of sulfate retention. This is one of the metabolites largely responsible for acidosis in the uremic patient.

From this quantitative study it is apparent that when the excretory function of the kidney fails, as measured by the endogenous creatinine clearance, a fairly predictable change occurs in the extracellular fluid concentrations of three important metabolites, which increase *pari-pasu* with the diminution of kidney function.

Summary

Interrelationships between serum creatinine, urea, sulfate and endogenous creatinine clearance have been described by means of empirical equations. They indicate that the metabolites studied follow the same mathematical law when expressed as functions of the clearance. Derivations from these fundamental equations show interesting relationships among several parameters of renal function.

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THYROID ADRENAL INTERACTION ON THE URINARY EXCRETION OF CREATINE IN THE RAT

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THE increase of urinary creatine is one of the most constant signs in hyperthyroidism; in fact, it has been observed, for a long time, that the administration of thyroid extract leads to a rise in creatine excretion in the urine ([3, 8, 9, 12, 15, 24]). Later on, this observation has been confirmed and a similar effect was obtained with TSH, but not with growth hormone or ACTH ([31]). Hyperthyroid patients both with exophthalmic goitre or with toxic adenoma, show a high incidence of increased creatinuria ([23]).

Although correlation between creatinuria and the calorigenic action of thyroxine has been sought, no clear direct relationship between them could be established ([6, 7, 23, 32]). In addition, a few cases have been described where the basal metabolic rate seemed to be almost inversely proportional to the urinary creatine ([12]).

Although there appears to be no direct relation between magnitude of oxygen consumption and creatinuria, yet decrease or absence of creatine excretion has been observed in hypothyroidism ([19, 29, 34, 38]). By giving thyroxine to hypothyroid patients or animals, their creatinuria rises even before the metabolic rate reaches its normal level ([19, 29, 34, 35, 38]).

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It is also worthwhile mentioning that the creatinemia of thyrotoxic subjects is high ([40, 46]); the altered creatine values in the serum and the urine return to normal with iodine treatment ([24, 28, 38, 40, 43]) or with thiouracil or methylthiouracil employed as antithyroid drugs ([2, 30, 36]).

A close interrelationship between the action of the thyroid and the adrenals has been pointed out in several occasions. Thus, it was observed that thyroxine does not increase the oxygen consumption in adrenalectomized animals, while their survival is reduced ([20, 21, 22]). Besides, when thyroxine is given to adrenalectomized rats, the rise in urinary nitrogen excretion does not occur as in normal cases ([21]). It has also been proved that the pituitary plays an important role in the thyroid-adrenal interaction ([11, 13, 17, 25, 26]).

It seemed therefore justified to study whether the adrenals participate in the increased creatine excretion that accompanies hyperthyroidism.

METHOD

Albino male rats from the laboratory stock were used. Their weight ranged from 80 to 120 g at the beginning of the experiments. The animals were kept in individual metabolic cages in

a room at about 28° C for the whole of the observation period, and fed "ad-libitum" with ground diet. Water added with sodium chloride 1 % and sugar 5 % was offered for drinking. The urine was collected with thymol as preservative.

Food and water intake, as well as urinary volume and body weight, were measured daily. Oxygen consumption was estimated every day in a closed circuit apparatus at 28° C ([20]). As no previous fasting of animals could be set up, results will be referred to standard instead of basal conditions.

Adrenalectomy was performed through a lumbar incision and the pituitary was removed via a parapharyngeal approach ([13]); in both cases ether anaesthesia was used.

Creatine, creatinine and non protein nitrogen in the urine were determined. Creatinine was measured by the method of Hare ([16]) and urinary nitrogen, by micro Kjeldahl. The creatine values were obtained according to Borsook's method ([5]), slightly modified since transformation to creatinine was brought about by immersing the tubes with the samples in a boiling water bath for one hour.

L-sodium thyroxine (Glaxo) was injected subcutaneously in daily doses of 1 mg/100 g body weight, from the second day after adrenalectomy and from the 10th day after hypophysectomy. The steroids (*) were administered intramuscularly starting from the day the adrenals or pituitary were removed, in daily doses of 1 mg. ACTH (Armour LA-I-A) was administered in amounts equivalent to 100 μg per day in a mixture of 5 % bee's wax in sunflower seed oil ([18]).

The results shown in the graphs were plotted with the mean values of each group. The standard error was obtained with the formula

$$\sigma = \sqrt{\frac{\Sigma\, d^2}{n\ (n-1)}}$$

Results

Action of thyroxine in intact rats.

As can be seen in Fig. 1, in intact rats injected with saline as control, the urinary creatine excretion was constantly low (0.35 mg/24 hr). When thyroxine was given to a group of intact animals, creatinuria increased, reaching values over 2 mg/24 hr in about 3 or 4 days of treatment.

In Fig. 1 the creatinine elimination of intact rats can also be seen. The curve shows that there is a slow and regular ascent of the urinary level of creatinine. Thus, while the initial values were about 2 mg/24 hr, at the end of the observation period they reached 2.5 to 3 mg/24 hr. Apparently the increase of creatinine in the urine is dependent on growth because when the results are expressed per 100 g body weight, the values remain constant at about 2 mg/24 hr. In Fig. 1 it can also be observed that thyroxine did not modify creatininuria substantially during the experimental period. It would also be worthwhile emphasizing the constancy of the creatinine excretion in thyroxinized rats, in which growth, expressed by their body weight, was about 10 % lower than that of their controls.

Action of thyroxine in adrenalectomized rats.

Creatinuria in adrenalectomized animals, Fig. 2, increased from 0.4 mg/24 hr to 1.5-2 mg/24 hr. When adrenalectomized rats were treated with thyroxine, creatinuria rose from the day following the first injection. The mortality of these animals started after the

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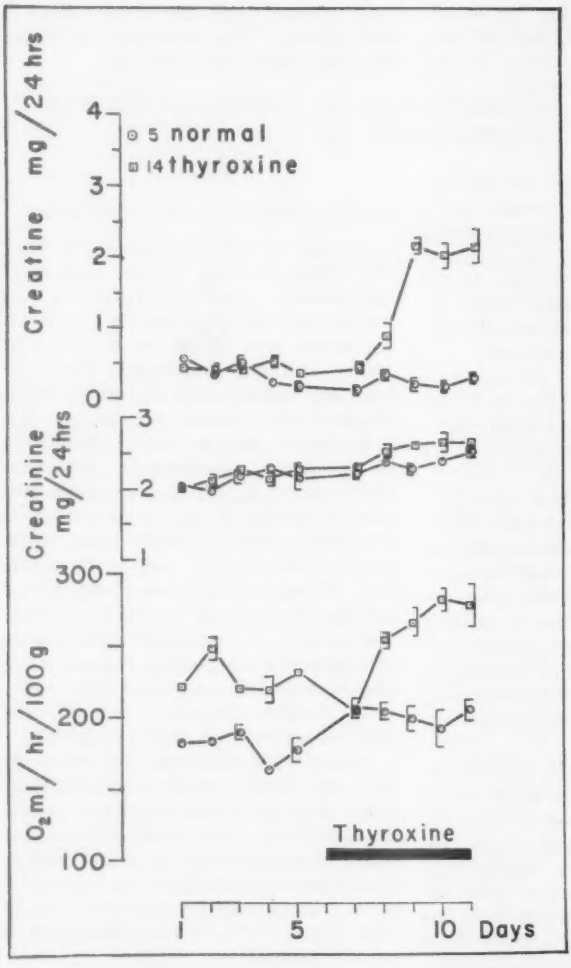

FIG. 1.—*Action of thyroxine on the urinary excretion of creatine and of creatinine and on the oxygen consumption of intact rats. The number of cases considered is shown on the upper left hand of the figure. The standard error of the mean is shown by the brackets. Description in the text.*

third dose of thyroxine and was always preceded by a marked rise of creatinuria, surpassing 6 mg/24 hr. Fig. 2 was drawn by averaging only the results of those animals which survived the complete period of observation; in spite of this, the dispersion of the values becomes greater as administration of thyroxine proceeds.

On the other hand, it can be appreciated on Fig. 2 that normal creatinine excretion did not change after adrenalectomy or thyroxine administration to these animals.

Action of thyroxine on hypophysectomized rats.

Removal of the pituitary gland led to a transitory increment of creatinuria. For this reason about ten days

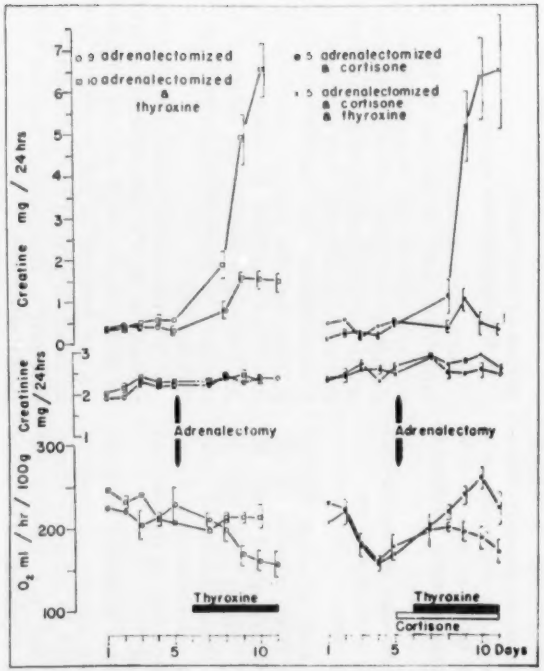

FIG. 2. — *Action of thyroxine on the urinary excretion of creatine and of creatinine and on the oxygen consumption. On the left hand, adrenalectomized rats; on the right hand, adrenalectomized rats injected with cortisone. The number of cases considered is shown on the upper left hand of the figure. The standard error of the mean is shown by the brackets. Description in the text.*

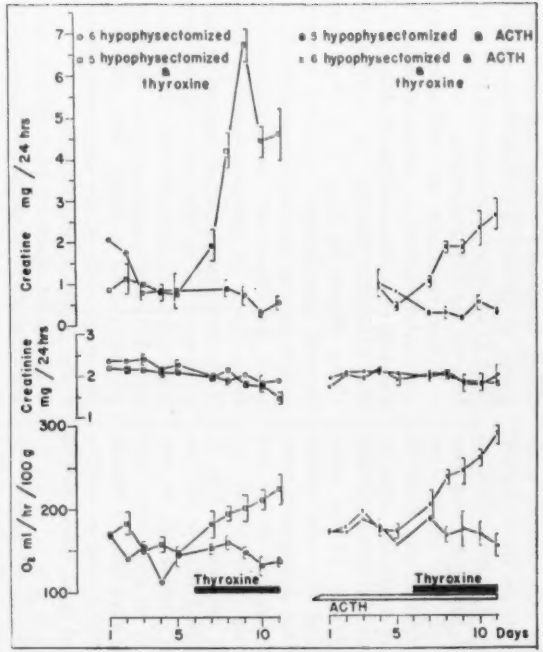

FIG. 3. — *Action of thyroxine on the urinary excretion of creatine and of creatinine and on the oxygen consumption. On the left hand, hypophysectomized rats; on the right hand, hypophysectomized rats injected with ACTH. The number of cases considered is shown on the upper left hand of the figure. The standard error of the mean is shown by the brackets. Description in the text.*

elapsed before the experiments were continued, so as to obtain stable values. As shown in Fig. 3, creatinuria of hypophysectomized rats remained low during the rest of the observation period. When thyroxine was given to a group of these animals, creatine excretion started to rise from the day following the beginning of the treatment; on the third day the values reached 6-7 mg/24 hr. Afterwards there was a certain decrease, and on the last day the creatinuria was about 4.5 mg/24 hr. In the records of Fig. 3, the animals who died during the experiment were not considered. It may be of some interest to state that high creatinuria values were always found, and thus death, in this case as well as in adrenalectomized ones, could almost be foretold.

On the other hand, creatinine elimination in the urine is similar at first to that of the intact controls; later the curve shows a slight decrease. Nevertheless, there are no apparent differences between the hypophysectomized rats receiving thyroxine with those not receiving the drug (Fig. 3). These animals did not show growth; therefore, if the results are expressed as a function of the body weight, a slow diminution of creatininuria persists.

Action of cortisone and hydrocortisone in adrenalectomized and hypophysectomized rats.

In order to study whether the deficiency derived from removal of the adrenals or pituitary gland could be compensated by giving cortisone or hydrocortisone, these substances were administered to adrenalectomized and hypophysectomized rats by the method already explained.

In the first place it should be pointed out that both steroids, in doses of 1 mg/day, injected to intact or sham-operated rats, did not modify the urinary creatine excretion. The same thing happened when cortisone was given to control adrenalectomized (Fig. 2) or hypophysectomized rats that had no further treatment.

When cortisone or hydrocortisone were administered to adrenalectomized animals simultaneously with thyroxine, the obtained results are similar to those shown in Fig. 2. It was considered unnecessary to draw a special graph for the action of hydrocortisone, since the effects are undistinguishable from those in adrenalectomized rats. Thus creatinuria due to thyroxine was as intense as in adrenalectomized rats which had no steroid-substitution therapy. Likewise, cortisone given to hypophysectomized animals, did not vary the magnitude of the increased creatinuria induced by thyroxine.

It should again be pointed out that the changes in creatine excretion were not accompanied by modifications in creatininuria, nor did the cortisone or hydrocortisone, in the doses used in the present experiments, alter the elimination of creatinine without accessory treatment.

Action of ACTH in hypophysectomized rats.

Since there appeared to exist a certain discrepancy in the results, because the creatininuria was exaggerated when thyroxine was given to adrenalectomized or hypophysectomized animals, and replacement therapy with cortisone or hydrocortisone was unable to compensate for the adrenal insufficiency, it seemed justified to study whether ACTH might modify the excretion of creatine in hypophysectomized-hyperthyroid rats.

It was considered important that the amount of ACTH administered should be sufficient to avoid adrenal atrophy due to removal of the pituitary, and to induce hypertrophy of the glands as obtained in thyroxinized animals with intact pituitaries.

TABLE I

Body and adrenal weight changes induced by thyroxine in normal and hypophysectomized rats

Groups	N° of animals	Body weight (g)	Adrenal weight (mg)	% changes
Normal	7	112	26·3 ± 1·3	0
Hypophysectomized (Hp)	6	101	13·6 ± 0·8	− 48
Thyroxine (Th)	8	94	30·1 ± 1·3	+ 14
Hp + Th	5	88	14·9 ± 0·6	− 43
Hp + ACTH	5	96	33·7 ± 2·4	+ 28
Hp + ACTH + Th	6	82	36·6 ± 4·2	+ 39

Values obtained the last day of the experiment.
Description in the text.

Preliminary experiments concerning the amount of ACTH required to fulfill these conditions, showed that about 100 μg per day, were adequate. The average adrenal gland weight of the different groups of rats, appear in Table I.

In the graphs of Fig. 3, the results of the experiments performed on two groups of hypophysectomized rats treated with ACTH are plotted. The group of rats injected with thyroxine, showed the same increase of creatine excretion in the urine as the intact hyperthyroid rats shown in Fig. 1; ACTH given to hypophysectomized animals did not modify by itself the urinary creatine elimination. A complementary series was performed in which ACTH was given to adrenalectomized rats; as expected, in this condition no protection to the hypercreatinuria due to thyroxine could be obtained.

It can also be appreciated in Fig. 3 that creatinine excretion remained unaltered when thyroxine and ACTH were administered.

Action of corticosterone in adrenalectomized rats.

Since ACTH was able to limit the creatine excretion in hypophysectomized rats injected with thyroxine, and

TABLE II

Comparative effects of thyroxine on the urinary excretion of NPN and of creatine in adrenalectomized or hypophysectomized rats treated with cortisone or ACTH, respectively

Groups	N° of animals	Total N mg/24hrs ingested	Total N mg/24hrs excreted	% N retention*	creatine N mg/24hrs	N ratio**
Intact	5	305	69	77·5	0·1	0·14
Intact + Thyroxine	14	369	98	73·5	0·69	0·70
Intact + Cortisone	5	267	88	67	0·11	0·12
Adrenalectomized (Adren)	9	300	105	65	0·48	0·45
Adren + Thyroxine	10	153	62	59·5	2·08	3·35
Adren + Cortisone	5	303	93	69	0·21	0·23
Adren + Cortisone + Thyroxine	5	251	101	60	1·99	1·97
Hypophysectomized (Hp)	6	138	53	61·5	0·18	0·34
Hp + Thyroxine	5	116	51	56	1·47	2·88
Hp + ACTH	5	179	70	61	0·1	0·14
Hp + ACTH + Thyroxine	3	242	96	60	0·78	0·81

$$(*)\ \%\ \text{N retention} = \frac{\text{Ingested N} - \text{Excreted N}}{\text{Ingested N}} \times 100$$

$$(**)\ \text{N ratio} = \frac{\text{Creatine N}}{\text{Total urinary N}} \times 100$$

Description in the text.

since cortisone or hydrocortisone were ineffective as substitutes of the adrenocortical function, it seemed interesting to investigate whether corticosterone, the main secretion of the adrenals in the rat, would be effective in lessening the creatinuria of hyperthyroidism.

Corticosterone (1 mg/day) was administered to each of 7 adrenalectomized rats. When thyroxine was given to these animals, creatine in the urine rose to the same average level of the adrenalectomized group without replacement therapy. For this reason it was considered unnecessary to include a graph of this set of experiments. It should also be added that no modifications in creatinine excretion occurred.

Oxygen consumption and urinary nitrogen excretion.

When thyroxine was administered, the oxygen consumption increased in all groups except in the adrenalectomized rats without additional steroid treatment. When adrenalectomized rats were injected with cortisone, hydrocortisone or corticosterone, thyroxine led to a rise in oxygen consumption similar to that observed in intact controls (Fig. 1 and 2). In Fig. 3 the relative independence between oxygen consumption and creatinuria can be appreciated. In fact when thyroxine was given to 2 groups of hypophysectomized animals with and without ACTH treatment, a very similar rise in oxygen consumption was produced in both of them while creatinuria was considerably more intense in hypophysectomized rats than in those also receiving ACTH; the latter behaved very much like intact animals injected with thyroxine.

In these experiments no precautions were taken in pair feeding the rats of the different groups, and although nothing definite can be inferred about their nitrogen balance, it can be noticed in Table II that in every case a positive nitrogen balance was maintained. This fact confirms other reports where it had been stated that in rats nitrogen equilibrium is reached very exceptionally.

Since in adrenalectomized rats there was an increase in the total urinary nitrogen excretion, the balance, expressed as the percentage of nitrogen retention, was less positive. Almost the same can be said for the hypophysectomized animals. When thyroxine was injected to adrenalectomized or hypophysectomized rats, the decrease in the percentage of nitrogen retention was of the same order of magnitude whether additional cortisone or ACTH was given or not.

Therefore it can be inferred from Table II that there does not exist a direct correlation between nitrogen excretion and creatinuria. Thus, it can clearly be seen that the marked creatine excretion (expressed as creatine nitrogen), due to thyroxine administration in adrenalectomized animals gave a very high $\frac{\text{creatine N}}{\text{Total N}}$ ratio. For the same reason, this ratio was also increased when thyroxine was injected to hypophysectomized rats. By the simultaneous treatment of hypophysectomized rats with thyroxine and ACTH a ratio quite similar to that of hyperthyroid intact rats was obtained. On the other hand, when cortisone was given together with thyroxine to adrenalectomized animals, the ratio was also very high; but the value was lower than the one obtained in only adrenalectomized or hypophysectomized rats treated with thyroxine. The difference appears to be due to an increase in the total nitrogen excreted, since cortisone by itself, as is well known, increases nitrogen elimination in the urine.

Discussion

It is a well known fact that in spontaneous and experimental hyperthyroidism creatine excretion in the urine is increased. In connection with this, at least two problems can be considered. One is the mechanisms by which hypercreatinuria is induced, and the other, is the role of endocrine interrelations in this metabolic disorder. This paper refers only to the thyroid-adrenal interaction.

It has already been established that the increment in oxygen consumption induced by thyroxine fails to appear in the absence of the adrenals ([20, 21]); besides, the increased urinary nitrogen excretion that accompanies thyrotoxi-

cosis is not observed when the adrenals are removed ([21]). On the other hand, the survival of adrenalectomized animals is shortened when thyroxine is administered ([22]). The reactions to thyroxine can be returned to normal in adrenalectomized rats when a cortisone-replacement treatment is added ([22]). Furthermore, when intact rats are thyroxinized the size of the adrenals increases and its content in ascorbic acid diminishes ([26, 13, 37]). These effects were interpreted as an adaptation reaction to greater corticosteroid demands that require the hypophysis for their appearance; the influence of the pituitary is evidenced when it remains in its normal position ([25]) or transplanted to the eye ([42]).

The fact that the increment in creatinuria of adrenalectomized rats is enhanced in comparison to the level reached by intact rats when thyroxine is given, makes it likely that in some way the adrenal antagonizes the effect of thyroxine on the urinary elimination of creatine. The sparing action of the adrenals on the creatine lost by the kidney is evident, since adrenalectomized animals without further treatment showed a rise in creatinuria; besides, in Addisonian patients, creatinuria has already been reported ([14]). The protective action of the adrenal glands becomes apparent only if the pituitary remains intact. This might be the explanation why hypophysectomized or adrenalectomized rats raise their creatinuria to similar levels when hyperthyroidism is induced. It seems unquestionable, therefore, that creatinuria of hyperthyroidism can be controlled, to a certain degree, by an increment of the adrenal function. The pituitary mediation is clearly shown in the experiments with hypophysectomized animals that had a supplementary treatment with ACTH. In these conditions, the response to thyroxine was a limitation in the creatine excretion, similar to the level reached in intact animals. The activation of the adrenals by ACTH cannot be substituted in the rat by cortisone, hydrocortisone or corticosterone, although this replacement therapy is efficient in other aspects of the thyroid-adrenal interrelationships. Thus, normal calorigenic response, increase in total urinary nitrogen, and survival of adrenalectomized rats were obtained when the above mentioned steroids were given together with thyroxine.

The disagreement between the sparing action of ACTH and the inefficacy of the adrenal steroids on the creatine excretion could be explained by assuming that the response of the adrenal to corticotrophin releases from the gland another substance that has not been identified. A plausible explanation that can be offered is that ACTH maintains a suitable structure of the gland, enabling it to react to another stimulant that would put in action some mineralocorticosteroid. This hypothesis is at present under study and the results will soon be reported.

The relative independence between metabolic rate and creatinuria should be emphasized. In spite of the references stating that there exists a certain discrepancy between oxygen consumption and urinary creatine level, there is no doubt that creatinuria rises when the general metabolism increases ([23, 32, 6, 7, 35]), and diminishes when the metabolic activity slows down ([19, 29, 35, 38]). In this paper evidence is given that the changes in urinary creatine and oxygen consumption may coincide but are in fact independent; for instance, hyperthyroidism induced in adrenalectomized rats did not show a definite rise in oxygen consumption; and it is precisely in this condition that creatinuria reached its maximum level. In this circumstance the parallelism that generally exists between

increment in urinary non-protein nitrogen and in creatine disappears; thus, in adrenalectomized animals, thyroxine did not increase the total urinary nitrogen excretion although creatinuria was extremely high.

The experiments reported here give no information about the mechanisms governing the creatinuria when thyroxine is administered. It is most likely that some hormonal interaction occurs at muscular level, where hyperthyroidism is accompanied by a diminution of the creatine and phosphocreatine content ([43, 1, 4, 39, 44, 45]). It has been postulated that in hyperthyroidism the level of muscular phosphocreatine would be diminished, probably due to a muscular defect in the ability to store and utilize the creatine. In this condition, there would exist a greater demand for steroid hormones and thus a state of relative insufficiency would be established ([39]).

It has recently been shown that thyroxine has an inhibitory effect on the creatine incorporation into the muscle ([10]), and, since its synthesis is accelerated ([10]), a greater load would be offered to the kidneys, thus providing an explanation for the creatinuria. This mechanism has already been suggested as a tentative explanation of the creatinuria in hyperthyroid subjects, in which renal tests showed that the tubular reabsorption was normal. It is known that when creatine surpasses the reabsorption capacity creatinuria develops ([45, 46, 33]). Therefore, creatinuria by itself should not be regarded as a sign of tubular deficiency, although lesions have been observed after prolonged thyroxine treatment in rats ([27, 41]).

Evidences have been presented confirming that excretion of creatinine is constant even when thyroxine is given ([10]). These results appear to be in contradiction with a previous communication in which a certain parallelism between creatine and creatinine was stated ([41]). However it should be considered that in the experiments described in that paper the increase in creatinuria appeared after the fifth day of thyroxine treatment, when urinary creatine had already reached its maximal value. On the other hand, there are many observations in which an increment of creatinuria with a simultaneous diminution of the plasmatic and urinary levels of creatinine in hyperthyroidism, has been established ([24, 35, 43-45]).

It would be interesting to localize the site where the thyroid-adrenal interaction takes place, since it would help to understand the mechanisms of the metabolic alterations produced by the thyroid as well as of many other modifications of the homeostasis in which there is a rise in the urinary excretion of creatine.

Summary

Administration of thyroxine for five days to rats led to a marked rise in creatinuria, reaching about five times the normal values. Creatinuria also increased about four times after adrenalectomy. When thyroxine was given to adrenalectomized animals, creatinuria rose to about 13 times the normal level. Thyroxine administered to hypophysectomized rats, produced creatinuria of about 10 times that of the respective controls.

The rise in creatinuria was the same when hyperthyroidism was induced in adrenalectomized or hypophysectomized rats, whether supplementary treatment with cortisone, hydrocortisone or corticosterone was given or not. When hypophysectomized animals were injected with ACTH, the creatine response to thyroxine was similar to that of intact controls.

The modifications of creatinuria were independent of the urinary ex-

cretion of creatinine, of the nitrogen elimination and of the changes in the oxygen consumption due to thyroxine.

From the above facts it can therefore be concluded that the increase of creatine excretion in hyperthyroidism can be influenced by the adrenal glands, although the substance responsible for this action has not yet been identified. The importance of endocrine interaction in the regulation of creatine metabolism is discussed.

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ROLE OF THE ADENO-HYPOPHYSIS IN THE ADRENAL HYPERTROPHY OF RATS WITH EXPERIMENTAL HYPERTHYROIDISM

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In thyrotoxicosis the whole endocrine system should be considered as a part of the total mechanism mobilized to maintain the homeostasis; for instance, the cortical hormones, have been shown to have an effect in this condition. If thyroxine is given to adrenalectomized rats and the oxygen consumption or urinary nitrogen excretion is studied, no change is seen unless the adrenal cortical deficiency is adequately treated ([13, 14]). It has also been found that oxycorticosteroids are the most effective in restoring the reacting capacity of adrenalectomized animals to thyroxine. Furthermore, when the treatment with thyroxine is intensified, it is necessary to increase the corticosteroid supply ([13]).

It is also known that in experimental or spontaneous hyperthyroidism the adrenal cortex becomes hypertrophied. In this hypertrophy microscopic changes are observed, such as lipoid depletion, decrease of the glomerular and increase of the reticular and fascicular zones. Chemical changes are also present, such as adrenal ascorbic acid depletion and raised levels of plasma corticosteroids and urinary ketosteroids. On the other hand, signs of adrenal hypofunction have been described in hypothyroidism ([1, 6, 16, 17, 19]).

The close relationship existing between thyroid and adreno-cortical activity has been studied by many authors ([7, 10, 11, 12, 16, 20]). It has been observed that the characteristic change in adrenal weight described for intact animals when thyroxine is given does not occur in hypophysectomized rats ([7]). This led us to explore the possibility that the adrenal reaction following thyroxine is mediated through the anterior pituitary gland. For this purpose, adrenal modifications, induced by thyroxine in hypophysectomized rats, with and without anteropituitary transplant in the eye, were studied.

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METHOD

White adult female rats of mixed breeding whose body weight is shown under Results were used. Oxygen consumption was measured daily, or every other day, by a method already described ([14]). Hypophysectomy was performed via a modified parapharyngeal approach (8). Control animals (sham-operated) were subjected to the same

surgical manipulations but the pituitary gland was not removed. Drinking water plus 5 % sugar was given. The animals were kept in a room at a constant temperature of 28 ± 1° C.

Pituitary grafts were performed 9-12 days after hypophysectomy. Female rats, approximately one month old, weighing 35-40 g were used as donors. The donors were exanguinated under light ether anesthesia, the base of the skull was opened an the pituitary was aseptically withdrawn with a trocar. A small incision of 1-2 mm was made in the corneal rim of the hypophysectomized animals anaesthetized with ether. After removing the lens and vitreous, the trocar was introduced to the opposite side of the eye, and the pituitary was gently placed in position.

Glaxo sodium l-thyroxine was daily administered by subcutaneous injections in dose of 0.5 mg/100 g of body weight. Control rats were injected with saline solution. Vaginal smears gave an index of the estrus cycle. Adrenal, thyroid glands and kidneys were weighed at autopsy. The sella turca was thoroughly examined with a dissecting binocular microscope. In some cases, histological examination of serial sections was also made. The report includes only data from those animals which proved to have total hypophysectomy at autopsy.

The eyes with transplanted hypophysis were fixed in Zenker-formol solution for 5 hours; afterwards they were embedded in paraffin and sections of seven microns in thickness were made. These were stained either with Harris hematoxylin and eosin or with azocarmine and aniline blue.

The adrenals were placed in 10 % formalin and frozen sections were made. One section without staining was observed in glycerin gelatin using a polarizing microscope with crossed nicols and an additional section was stained with Sudan III.

The data were analyzed by the small sample "t" test, and mean differences were considered significant if the value of P was 0.01 or less.

Results

Experiment A. — In this experiment 10 sham-operated rats, whose initial average weight was 146 gm ± 0.67 (standard error of the mean) at the

Thyroxine action on hypophysectomized and hypophysectomized pituitary — grafted rats

GROUPS	N° of animals	Survival in days	Adrenal weight mg absolute	Adrenal weight mg per 100 g b.w.	Thyroid weight mg absolute	Thyroid weight mg per 100 g b.w.	Kidney weight mg absolute	Kidney weight mg per 100 g b.w.
Normal	5	—	47.1±1	28	21.0±75	12	1399±36	831
Thyroxine	5	—	71.1±3	45	18.3±1.7	11	1805 ±102	1152
Hypox 40 days	8	—	8.4± .3	7.7	8.9± .5	8	764± 16	711
Hypox 12 days + thyroxine	12	6.6± .06	15.9± .5	15 .2	11.7± .5	11	1025 ± 29	978
Hypox 40 days + thyroxine	9	7.7± .5	8.5± .4	9 .5	7.7± .3	9	862 ± 34	963
Grafted	5	—	10.3± .4	10.6	10.0± .7	8	801 ± 27	690
Grafted + thyroxine	10	11.6± .25	13.5± .5	13 .6	11.3± .8	11	1198 ± 29	1209

Mean values are followed by the standard error of the mean $\sigma = \sqrt{\frac{\Sigma\, d^2}{n\,(n-1)}}$

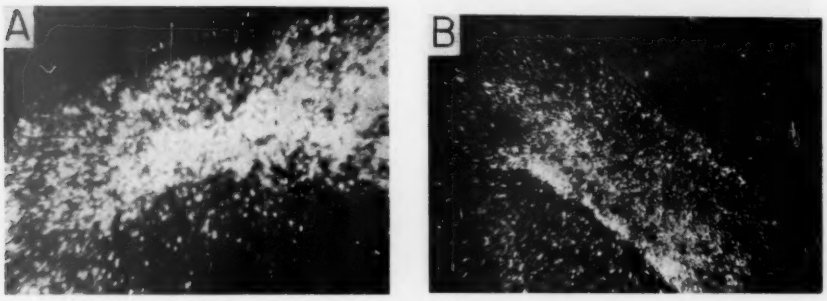
time of operation, were used. Of these, five rats received thyroxine for 6-11 days and the other five for 10-14 days. These treatment periods coincided with those of the hypophysectomized animals. In both groups a definite decrease in body weight was observed, and the expected oxygen consumption increase was seen. The adrenal, the thyroid and the kidney were weighed at the end of the treatment with thyroxine. The results shown in Table 1 refer only to the 5 rats that received thyroxine for 6-11 days. The rats treated with thyroxine during 10-14 days showed similar changes. For this table the normal values were obtained from sham-operated animals of approximately the same age. It is evident that thyroxine causes an increase in adrenal and kidney weight, and a decrease in the size of the thyroid gland.

In the thyroxine-treated sham-operated rats, the histological section of the adrenals showed an enlarged cortex with an increase of the fascicular and reticular zones where the cells showed reduced and finer lipoid inclusions. These changes in the microscopical picture are shown in Figure 1 where comparison with a gland of an animal not treated with thyroxine can be seen.

Experiment B. — In this experiment hypophysectomized animals were divided in two groups: one was treated with thyroxine and the controls received saline solution instead. Hypophysectomized rats injected with thyroxine were grouped so that in some of them the thyroxine administration began approximately 12 days and in the others 40 days after hypophysectomy was performed. The controls were all injected with saline solution at comparable times. The results appear in Figure 2.

As expected, the body weight and oxygen consumption were decreased in the hypophysectomized rats, when compared to the controls of experiment A. Treatment with thyroxine starting at 12 and 40 days after hypophysectomy, produced a slight drop in body weight. In Figure 2 it can also be observed that in hypophysectomized rats of 12 days, the oxygen consumption increase starts at the same time as in the sham-operated controls, whereas the hypophysectomized rats of 40 days show a delay of about 60 hours to increase the oxygen consumption after the first injection of thyroxine. In comparison with the hypophysectomized controls and sham-operated animals, hypophysectomized rats treated with thyroxine had a shorter survival time. When thyroxine was given 12 days after hypophysectomy, the rats showed a mean survival time of 6.6

FIG. 1. — *Lipoid inclusions under polarizing microscopy. A. Normal adrenal cortex. 130 ×. B. Adrenal cortex from a hyperthyroid rat. 45 ×. Description in text.*



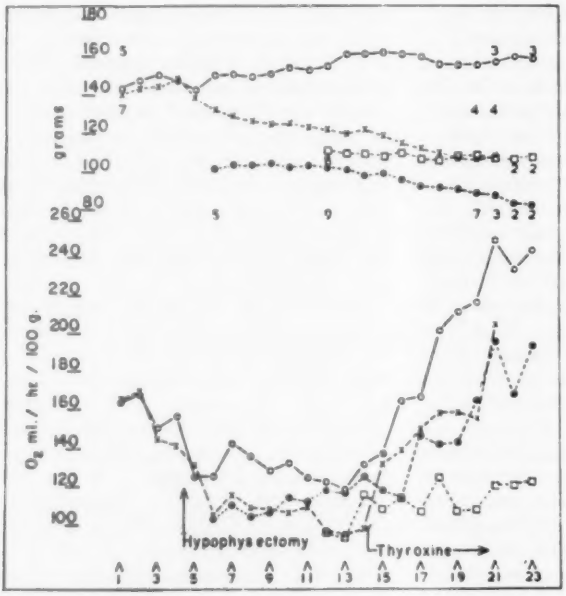
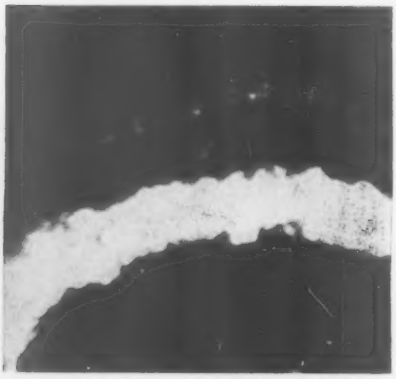


FIG. 2. — *Thyroxine action on body weight and oxygen consumption of rats. Symbols represent:* ○ — ○ = *Sham-operated injected with thyroxine.* × - - - × = *12 day-hypophysectomized, injected with thyroxine.* ● - - - ● = *40 day-hypophysectomized, injected with thyroxine.* □ - - □ = *40 day-hypophysectomized, injected with saline solution. The number of cases considered in each point are indicated by the figures. Abscissae, days. Description in text.*

days, and in those receiving thyroxine after 40 days of the operation, a similar survival was obtained (Table I). Eight hypophysectomized rats injected with saline solution were sacrificed so as to compare them with the corresponding thyroxine injected animals. Table I shows the weight of the adrenals, thyroid and kidneys in the different groups of animals studied. No difference is observed in the weight of the adrenals in hypophysectomized rats, whether thyroxine was given or not. It is interesting to compare the weight of these glands with those of the sham-operated thyroxinized rats. It can also be observed that the decrease in size of the thyroid gland, produced by hypophysectomy, is not modified by thyroxine. On the other hand, although hypophysectomy determines a decrease in renal size, thyroxine induces a slight increase in the absolute weight of the kidneys, but without reaching the normal levels. It is obviously concluded from Table 1, that the decrease in the size of the organs is somewhat correlated with the time of hypophysectomy.

FIG. 3. — *Lipoid inclusions of the adrenal cortex of a hypophysectomized rat receiving thyroxine. 130* ×. *Description in text.*



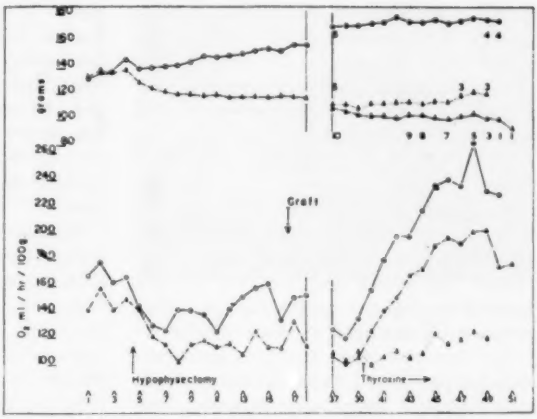


FIG. 4. — *Thyroxine action on body weight and oxygen consumption of rats. Symbols represent:* ○ — ○ = *Sham-operated injected with thyroxine.* ▼ - . - ▼ = *Hypophysectomized-grafted, injected with thyroxine.* △ - - - △ = *Hypophysectomized-grafted, injected with saline solution. The number of cases considered in each point are indicated by the figures. Abscissae, days. Description in text.*

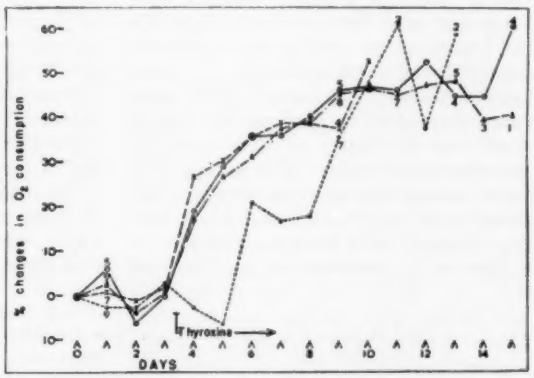


FIG. 5. — *Action of thyroxine on the oxygen consumption of rats. Symbols represent:* ○ — ○ = *Sham-operated.* ▼ - . - ▼ = *Hypophysectomized - grafted.* × - - - × = *12 day-hypophysectomized.* ● - - - ● = *40 day-hypophysectomized. Numbers of animals considered in each point are indicated by the figures. Description in text.*

There is no great microscopic difference between sections of the adrenals from hypophysectomized rats injected with thyroxine or saline solution. For this reason in Figure 3 there appears only one microphotograph corresponding to the adrenal of a 40 day-hypophysectomized rat that received thyroxine. The reticular and fascicular zones becomes atrophic and thickness of the glomerular zone is apparently normal. In hypophysectomized animals of 40 days the birefringence is divided into two zones that can be appreciated in the microphotograph of Figure 3. The outer zone has a smaller amount of lipoid inclusions than the interzone, whereas in 12 day-hypophysectomized animals the glands are not so atrophic and the microscopic examination reveals a greater quantity of lipids distributed more homogeneously between the reticular and fascicular zones.

Experiment C. — In this experimental group a pituitary graft was implanted in the eye, 9 to 12 days after hypophysectomy. During this time the weight curve, oxygen consumption and vaginal smears were observed. For the final results only 15 animals, presenting at autopsy histological evidence of total hypophysectomy and functional

graft, were considered. The vaginal smears were somewhat indicative as to the success of the graft. Often, the successful grafts produced signs of proestrus or estrus, while anestrus and metaestrus phases were observed in non-implanted hypophysectomized animals.

In some grafted rats, I^{131} uptake and release by the thyroid was studied. In this way some information was obtained regarding the activity of the transplant and the production of thyrotrophin. A detailed account of the results obtained from these experiments will be published in a separate paper.

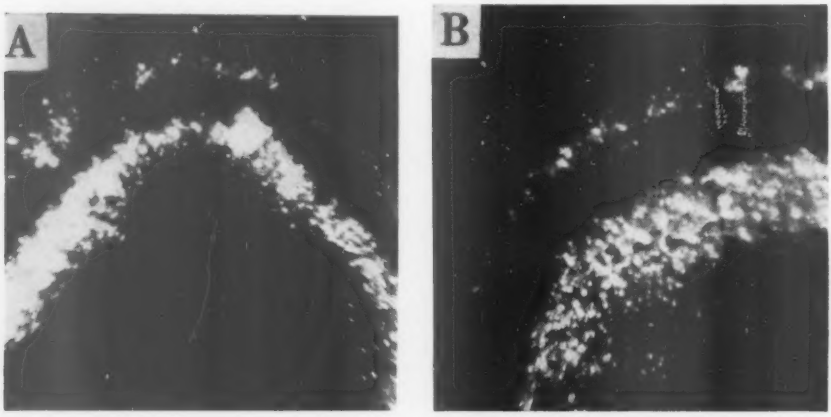
If Figure 2 and 4 are compared, one can see that the curves of oxygen consumption and increase in weight do not show great differences between hypophysectomized-grafted and non-grafted animals. Therefore, these measurements have no informative value regarding the degree of activity of the transplanted pituitary. The grafted rats were regrouped and thyroxine was administered to 10 animals, while the remaining 5 were kept as controls.

Figure 5 describes a comparative study of the changes induced by thyroxine on oxygen consumption. The administration of thyroxine to 40 day-hypophysectomized-grafted rats produced an increase in oxygen consumption about 12 hours after the first injection of the hormone, while in the non-grafted animals it began only after 60 hours.

Another aspect in this experimental series refers to the survival of animals injected with thyroxine. The maximum survival of grafted rats was 14 days after the beginning of the thyroxine treatment, with an average time of about 11.6 days. ($P < 0.01$ in comparison with the hypophysectomized rats.) No data regarding the survival of grafted animals not injected with thyroxine are reported since these were sacrificed in order to have an exact control of the weight and histological aspect of the organs.

In Table I it can be seen that the size of the adrenals, previously reduced by hypophysectomy, is slightly increased in transplanted animals ($P < 0.01$). Thus, from this point of view the transplant showed to be active, al-

FIG. 6. — *Lipoid inclusions of adrenal cortex from hypophysectomized-grafted rats. A. Without treatment. B. Treated with thyroxine. 130 ×. Description in text.*



though the adrenal gland maintained their weight below the normal values. The adrenals of hypophysectomized-grafted animals treated with thyroxine, weighed more than those of the animals injected only with saline solution ($P < 0.01$). Furthermore, as shown also in Table I, hypophysectomy produced a remarkable atrophy of the thyroid. On the other hand, the pituitary transplant determined an increase in the size of the thyroid gland. The administration of thyroxine did not modify the increase obtained with the transplant.

The size of the kidney, reduced (Table I) by hypophysectomy, was not modified by the pituitary transplant "per se", whereas it increased when thyroxine was given to these rats.

In the adrenals of rats with pituitary grafts there was a diminished thickness of the cortex with lipoid inclusions mainly present in the fascicular zone and, in minor quantities, in the glomerular region (Fig. 6). In transplanted rats the histological picture of the adrenals did not show great changes after the treatment with thyroxine except a certain rarefaction of the lipoid inclusions. On the other hand, in the histological sections of pituitary transplants, the existence of numerous blood vessels and glandular nests of normal appearance were found. The nests presented the three types of cells, variable in number and proportion, but principally chromophobe elements mixed with cells derived from the inflammatory reaction (Fig. 7).

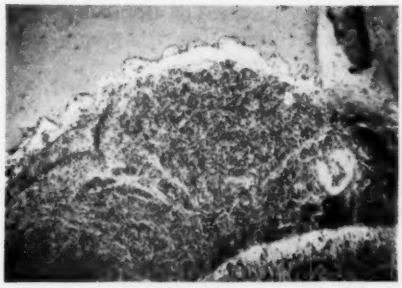


FIG. 7.—*Pituitary graft in a hypophysectomized rat. 130 ×. Description in text.*

DISCUSSION

In these studies further evidences of an hypertrophy of the adrenal gland in experimental thyrotoxicosis are given.

Does the adrenal hypertrophy or atrophy present in hyper or hypothyroidism actually represent a condition of absolute adrenal hyper or hypoactivity? The answer must consider not only the direct effect of thyroxine on the adrenal cortex but also the body requirements in corticosteroids. Conceivably, the magnitude of the body requirements for corticosteroids could determine the rate of the steroid production.

By measuring the 17-hydroxicorticosterone in man, it has been shown that the plasma level decreases faster in hyperthyroid than in normal patients ([17]). Recently it has been shown that alterations in thyroid function influence the hepatic reduction of cortisone. These effects provide an enzymatic basis for the alterations in biological half life of adrenal steroids observed in hyper and hypothyroidism([20]).

The enlargement of the gland, induced by thyroxine, has distinctive microscopic characteristics already ascribed, by other authors, as typical of the hyperfunction states ([6, 16]) and characterized by increase in thickness of the fascicular and reticular zones with lipoid depletion. The present experiments emphasize the importance that the changes in the morphological aspect of the adrenal cortex are accomplished by an indirect action of the thyroid hormone since, in the absence of the hypophysis there are no changes in the adrenal glandular weight or

histological picture, or lipoid distribution that follows the treatment with thyroxine.

However, in hypophysectomized animals with pituitary grafts, the adrenals grow in a slight but significant degree due to thyroxine administration. This fact indicates that thyroxine itself, or some of its metabolic products or substances formed as a consequence of its calorigenic action, could act directly on the adenohypophysis.

In regard to the calorigenic action induced by thyroxine, it is worthwhile remembering that rats receiving thyroxine twelve days after hypophysectomy responded in a similar way to sham-operated animals, namely, with an increase in oxygen consumption about 12 hours after the first injection. However, if thyroxine was given to rats that were hypophysectomized 40 days before, the metabolic response was obtained only about 60 hours after the first injection of thyroxine. On the other hand, when long term (40 days) hypophysectomized rats, that carried pituitary grafts, were treated with thyroxine, the increase in oxygen consumption started at a similar time as in normal or short term hypophysectomized animals (12 days).

No clear explanation can be given so far for the prolonged latency in the response to thyroxine of long term hypophysectomized rats. It could be due, at least in part, to the marked degree of adrenal atrophy, that would condition a low blood level of corticosteroids.

The remaining adrenal steroid secretion in the absence of the pituitary is probably due to release of these hormones, as an autonomic function of the adrenal gland, as it has been already suggested (14). Long term hypophysectomized animals, carrying pituitary grafts had increased adrenal gland weight when thyroxine was given and the survival time was prolonged, in comparison to the hypophysectomized non-implanted rats. These changes, in response to thyroxine administration might be due to a discharge of ACTH by the pituitary graft and thus a certain degree of command on the function of the adrenal gland could have again been obtained. It is known that pituitary grafts allow depletion of adrenal ascorbic acid in rats exposed to acute stress (4, 5, 15).

Furthermore, it has recently been reported that hypophysectomized rats with pituitary transplantation in the anterior chamber of the eye have adrenal weights significantly higher than those of the hypophysectomized ones (9, 15).

The finding that the renal weight increased when thyroxine was given to hypophysectomized animals bearing pituitary grafts seems to be interesting. The renal hypertrophy is in agreement with the hypothesis that relates the renotrophic activity to the intensity of protein metabolism (2). Therefore it could be postulated that some factor of pituitary origin is necessary for the full renotrophic action.

The present results tend to support the view that the increased requirements of corticosteroids brought up in experimental hyperthyroidism would be paid off, at least partially, by a direct mechanism acting on the adenohypophysis.

This conclusion does not exclude other possibilites of regulation of the complex pituitary reactions that might involve the hypothalamus. This part of the hypothesis is under study and a brief communication has been reported (18).

Summary

Thyroxine increases the oxygen consumption of hypophysectomized rats. The maximum increase in oxygen consumption expressed as a percentage of

the pre-thyroxine treatment levels is similar in control and hypophysectomized animals. The response of hypophysectomized rats to thyroxine, injected twelve days after hypophysectomy, is similar to that of control rats and becomes apparent within twelve hours; on the other hand, hypophysectomized rats injected with thyroxine forty days after operation show no increase in oxygen consumption till after sixty hours.

The survival of both groups of thyroxine-treated hypophysectomized rats, is short.

Hypophysectomized rats which had received an intraocular pituitary graft twelve days after operation, showed a rapid response to thyroxine (within twelve hours) injected forty days post-hypophysectomy. Survival in this group was significantly longer than in the non-grafted hypophysectomized rats.

The adrenals of hypophysectomized rats show progressive atrophy. Histologically, the reduction in size is more marked in the fascicular and reticular zones. Lipids, studied with polarized light microscope, tend to be distributed in two bands, in the fascicular and glomerular zones. In rats forty days after hypophysectomy birefringence is seen mainly in the fascicular zone.

The adrenals of hypophysectomized rats injected with thyroxine are similar to those of non-injected hypophysectomized animals.

In the hypophysectomized rats with an intraocular pituitary graft, atrophy of the adrenals is less marked. The lipids are more abundant and are distributed in two clearly separated bands. Thyroxine treatment of these animals further increases gland weight but does not produce any further change in the histological picture.

It is concluded that the adrenals of hypophysectomized rats continue to produce small quantities of corticosteroids and that thyroxine is only able to modify the adrenals when the anterior pituitary is present.

Acknowledgment

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VASCULAR RESPONSES TO ADRENALINE, NORADRENALINE AND ANGIOTENSIN IN HYPOTHERMIC DOGS

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THE present work was performed to study the possible changes associated with hypothermia in the cardiovascular responses to vasoactive drugs. Three sustances with pressor action, adrenaline, noradrenaline and angiotensin, were injected in hypothermic dogs and vascular reactivity was determined.

MATERIAL AND METHODS

Studies were carried out on 35 mongrel dogs weighing 8 to 12 kg, under pentobarbital anesthesia (35 mg/kg, intraperitoneally). The left femoral artery and the yugular vein were cannulated for continuous recording of blood pressure on a mercury manometer and for injection of drugs, respectively. The right carotid artery, was connected to a Hamilton type, rubber membrane manometer of proper natural frequency (60 cycles/sec) and sensitivity, through an indwelling needle (1.5 mm O.D.) and a lead tube. Optical recording was performed on a photographic kymograph, 2 meters apart from the membrane mirror, at a paper speed of [illegible] mm/sec. Tracheal intubation was performed and a resistance thermometer placed 10 cm into the rectum. In 6 dogs both vagii were cut and in 5, a ganglionic blocking agent was injected. In 10 dogs the drugs were tested in single injections and in doses of 1, 1.5, and 2 μg/kg for adrenaline (A) (*) and noradrenaline (NA) (**), and 0.1, 0.15, and 0.2 Goldblatt units/kg for angiotensin (Agtn) (***).

In the other 25 dogs, the drugs were constantly infused, after a priming dose of 1 μg/kg for A and NA, and of 0.2 Goldblatt units/kg for Agtn.

(*) This work was done during a fellowship of the "Asociación Argentina para el Progreso de las Ciencias". Present address: Instituto de Investigaciones Médicas, Universidad de Buenos Aires.

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(**) (A), l-adrenaline chloride (Parke Davis).

(***) (NA), l-arterenol bitartrate (Winthrop).

(****) (Agtn), synthetic angiotensin II, val-octapeptide-amide (Ciba).

Constant injection did not exceed the rate of 6 ml/min, at the dosage of 1.2 to 1.4 μg/kg/min for A, 0.9 to 1.1 μg/kg/min for NA, and of 0.5 to 0.7 Goldblatt units/kg/min for Agtn. Before and after testing the drugs, a bilateral carotid occlusion of 60 seconds duration was done, as a test for baroceptors activity. The dogs were then immersed in a water bath at 4° C. Cooling proceeded until rectal temperature reached 28-27° C; the animals were withdrawn from the water, dried and heated with an electric stove to prevent further spontaneous cooling; stabilization of rectal temperature at 25-24° C was thus obtained. Respiration was maintained with a bellows type pump. The drugs were again tested, but in a different order chosen at random. The variables were measured in the photographic record of the pressure pulses enlarged 3 times with an optical pantograph. The parameters determined were: systolic and diastolic pressures, heart rate, mean arterial pressure (by planimeter integration), stroke volume with the Hamilton-Remington method (4) and total peripheral resistance (TPR) (by dividing mean arterial pressure over cardiac output). The solutions of A and NA (100 μg/ml in 96 % alcohol) and of Agtn (75 Goldblatt units/ml in pH 9 buffer) were stored at −20° C until needed, and diluted when used in fresh 5 % glucose in water. Ansolysen (*), a ganglionic blocking agent (100 mg/ml) was stored at 0° C.

Results

Hemodynamic changes induced by hypothermia.

Mean arterial pressure was significantly lowered by hpyothermia: in intact dogs (36° C : 110 ± 18 mmHg; 25° C; 85 ± 22 mmHg), in vagotomized (36° C : 122 ± 24 mmHg; 25° C : 90 ± ± 19 mmHg) and in ganglion blocked dogs (36° C : 80 ± 11 mmHg; 25° C : 50 ± 5 mmHg).

Stroke volume was not significantly altered by hypothermia, in any of the groups measured, and no significant differences were found between them. (Intact, 36° C : 10.3 ± 2.2 ml; 25° C : 12.6 ± 5 ml) (Vagotomized, 36° C : 10.9 ± 2.0 ml; 25° C : 11.2 ± 1.8 ml) (Ganglion blocked, 36° C : 9.6 ± 1.3 : 25° C : 10.7 ± 1.2 ml).

Heart rate was significantly slower at 25° C in all the groups, thus lowering the cardiac output (intact 36° C : 1 340 ± 220 ml/min; 25° C : 634 ± 110 ml/min) (vagotomized, 36° C : 1 210 ± ± 196 ml/min; 25° C : 565 ± 75 ml/min), (ganglion blocked 36° C : 1 180 ± 170 ml/min; 25° C : 520 ± 60 ml/min). Heart rate was not uniformly

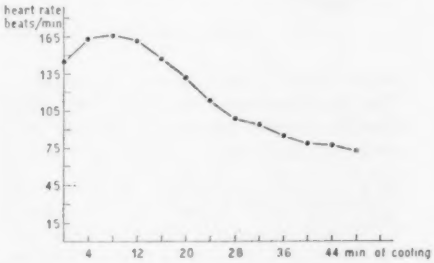

FIG. 1. — *Mean values of heart rate during cooling, in 10 anesthetized dogs, cooled by immersion in a water bath at 4° C.*

slowed by cooling (Fig. 1); at the beginning of it, an increase in rate, was observed and then, a pronounced decrease with a variable slope.

Total peripheral resistance (TPR) increased significantly in hypothermia in controls (36° C : 6 120 ± 1 450 dynes; 25° C : 10 075 ± 2 120 dynes), in vagotomized (36° C : 9 650 ± 2 320 dynes; 25° C : 12 730 ± 2 650 dynes) and in pentolinium-injected dogs (36° C : 4 320

(*) (Ansolysen), pentolinium tartrate (May & Baker).

± 850; 25° C : 8 630 ± 1 430 dynes). The ganglion blocked group departed from a significant lower level of resistance.

Responses to single injections.

It was observed that in hypothermia, the pressor responses to single rapid injections were significantly reduced for all the drugs (A 1 μg/kg, 36° C : 24 ± 4.5 mmHg; 25° C : 17.5 ± 3.2 mmHg), (NA 1 μg/kg, 36° C : 28 ± 3.7 mmHg; 25° C : 16 ± 2.9 mmHg), (Agtn 0.1 Goldblatt unit/kg 36° C : 19 ± 2.4 mmHg; 25° C : 23 ± 3.5 mmHg). The dose-response relation (Fig. 2) showed

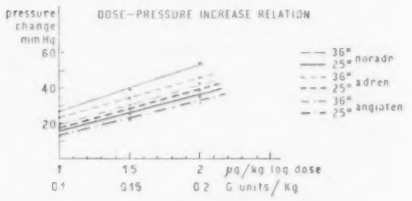

FIG. 2.—*Relationship between mean blood pressure increase and injected dose, in 10 intact dogs after single rapid injections of A, Na (1, 1.5 and 2 μg/kg) and Agtn (0.1, 0.15 and 0.2 Goldblatt units/kg). Thin lines: normothermic data. Heavy lines: hypothermic data. G. units — Goldblatt units.*

that while in normothermia NA gave the higher responses (followed by A and Agtn), in hypothermia A and NA gave similar increases in pressure (no significant differences), and both were higher than Agtn.

Stroke volume increased proportionally to the dose injected (from 1.8 ± ± 0.4 to 3.2 ± 0.5 ml with A; from 1.7 ± 0.3 to 3.9 ± 0.3 ml with NA; and from 1.2 ± 0.2 to 2.4 ± 0.5 ml with Agtn). During hypothermia these changes were not significantly different from those at 36° C.

Total peripheral resistance increased with augmenting dose (± 780 ± 240 to + 1 890 ± 605 dynes with A) (+ 890 ± 208 to + 2 070 ± 560 dynes with NA), (+ 640 ± 210 to + 1 760 ± 600 dynes with Agtn) and during hypothermia it was observed that resistance increased in a minor degree, but not significantly.

During normothermia, the time of onset and the time of duration of the pressor responses, were similar with the different drugs (time of onset in seconds: 7.8 ± 1.6 to 9.1 ± 1.7; time of duration in seconds: 98.6 ± 19 to 107 ± 19). In hypothermia both times were significantly longer, without difference between drugs (time of onset in seconds: 15.4 ± 2.8 to 21.2 ± 3.4; time of duration in seconds: 175 ± 31 to 210 ± 30).

Responses to constant infusions.

Responses to sustained injections of the drugs were studied in 25 dogs, 14 intact (controls), 6 vagotomized and 5 injected with pentolinium (ganglion-blocked).

Intact dogs showed moderate increases in pressure during normothermia at the dosage injected (36° C, A : 6 ± 3 mmHg; NA : 13 ± 4 mmHg; Agtn : 10 ± 2 mmHg), in hypothermia the pressor responses were significantly augmented, for all the drugs. The potentiating effect of cooling was more important in responses to A (25° C, A : 30 ± 2 mmHg; NA : 24 ± 3 mmHg; Agtn : 25 ± 4 mmHg).

In vagotomized dogs, pressor responses were higher than in controls during normothermia (36° C, A : 15 ± 2 mmHg; NA : 20 ± 5 mmHg; Agtn : 15 ± 3 mmHg), at 25° C the increase in pressure was not significantly different than before with NA and Agtn, but it was significantly higher with A (25° C, A : 30 ± 6 mmHg; NA : 22 ± 4 mmHg; Agtn : 17 ± 4 mmHg).

Ganglion blocked dogs during nor-

mothermia showed the highest increases in pressure after infusions, for all groups (36° C, A : 18 ± 4 mmHg; NA : 32 ± 6 mmHg; Agtn : 28 ± 7 mmHg); it was found that hypothermia did not alter significantly the magnitude of the response for any of the drugs tested (25° C, A : 29 ± 5 mmHg; NA : 35 ± 4 mmHg; Agtn : 30 ± 6 mmHg).

In normothermic dogs, stroke volume increased significantly after A in all groups without significant differences between them (+ 4.3 ± 0.5 ml). NA induced little increase in stroke volume, which was similar in intact and vagotomized dogs (1.5 ± 0.5 ml), and still smaller ones, in pentolinium-treated dogs (+ 0.80 ± 0.6 ml). Angiotensin gave no significant changes in stroke volume in any of the groups (0.6 ± 0.6 ml to 0.3 ± 0.6 ml). Hypothermia altered the responses for all the drugs. In control dogs, A gave lesser increases than at 36° C (+ 2.80 ± 0.5 ml), while NA and Agtn gave similar changes (NA : 1.6 ± 0.3; Agtn : 0.3 ± 0.5 ml). In vagotomized dogs all the infused sustances, diminished significantly the stroke volume, although Agtn in a major degree (A : − 0.5 ± 0.5 ml; NA : − 0.3 ± 0.7 ml; Agtn : − 2.2 ± 0.6 ml). In pentolinium-treated dogs it was observed a decrease in the stroke volume after the sustained injection, which was significant for A (− 1.8 ± 1.1 ml) and Agtn (− 2.4 ± 1.4 ml) and not for NA (− 0.9 ± 0.6 ml).

The changes in TPR induced by the pressor drugs, were significantly modified by cooling. Adrenaline diminished the resistance during normothermia in control (− 2 990 ± 1 500 dynes), vagotomized (− 3 970 ± 1 000 dynes) and ganglion-blocked dogs (− 2 000 ± 600 dynes). In hypothermia the same drug gave significant increases in TPR, in intact (+ 2 700 ± 1 500 dynes), vagotomized (+ 6 700 ± 1 600 dynes) and pentolinium-injected animals (+ 7 800 ± 2 200 dynes).

Noradrenaline increased the resistance during injection at 36° C, in intact animals (+ 800 ± 200 dynes) and more so in vagotomized (1 500 ± 500 dynes) and ganglion-blocked dogs (1 300 ± 570 dynes). The infusion in hypothermia determined a more pronounced increase, in control (+ 2 300 ± ± 1 200 dynes), and more so in vagotomized (+ 5 400 ± 2 000 dynes) and in pentolinium-injected dogs (+ 6 200 ± 1 950 dynes).

Angiotensin induced increases in resistance during normothermia, less in control dogs (+ 500 ± 200 dynes) than in vagotomized (+ 2 200 ± 1 200 dynes) or pentolinium-treated dogs (+ 3 500 ± 1 800 dynes). The resistance increases obtained during hypothermia were greater than those in normothermia, in control (+ 3 500 ± 900 dynes), vagotomized (+ 8 000 ± 2 000 dynes) and ganglion-blocked dogs (+ 8 900 ± ± 2 300 dynes).

Carotid occlusion test.

Bilateral carotid occlusion determined a significant rise in blood pressure during normothermia, which was greater in vagotomized animals (+ 32 ± 7 mmHg) than in control dogs (+ 26 ± 5 mmHg). Pentolinium-injected dogs did not show any change in mean blood pressure during occlusion.

Hypothermia diminished significantly the post-occlusion rise of mean blood pressure, in control (+ 11 ± 4 mmHg) and vagotomized dogs (14 ± 8 mmHg); the difference between groups was not significant.

Separate effects of priming and continuous doses.

The recorded blood pressure during sustained injection of the drugs was determined by the additive effect of priming over continuous dose. As the degree of summation could be altered

by hypothermia, in some cases we have performed separatedly the priming and the continuous doses. Then, the change in pressure resulting from each injection was added to the other from O time (injection time) and the record resulting from superposition, was compared with that recorded as usual (continuous following inmediately the priming dose). A representative experiment with NA, done with this procedure is shown in Fig. 3, performed in

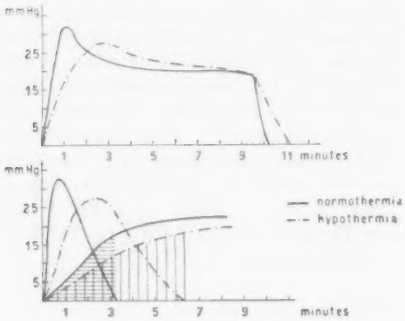

FIG. 3. — *Blood pressure changes in a single dog. Above: Mean arterial pressure increase, in normothermia (full line) and in hypothermia (dotted line); after the injection of a priming dose (NA:1 μg/kg) followed by the sustained injection during 10 minutes of NA at a rate of 1 μg/kg/min. Below: Mean arterial pressure increase, in normothermia (full line) and in hypothermia (dotted line), after the injection of a single rapid dose of NA 1 μg/kg), and then, after the sustained injection of Na at the rate of 1 μg/kg/min. The horizontal and vertical lines indicate the areas where the effect of the constant injection, is associated with that of the priming dose, if each separate effect is added from O time. (Horizontal lines: changes during normothermia; vertical lines; changes during hypothermia.)*

normothermia and hypothermia. It could be observed that in hypothermia the area in which priming is added to the continuous dose (vertical lines), is significantly greater than the normothermic area (horizontal lines).

Discussion

The reported diminished pressor responses to single injections in hypothermia, are in accordance with the data of other authors [1-3]. It was suggested [3] that this lowered response may be due to a decreased inotropic effect of drugs over the myocardium under hypothermia. However, cardiac contractility is augmented [17] or not changed [18] during hypothermia; and as we have observed, the stroke volume did not change significantly at 25° C. Other authors [12, 13] support the peripheral origin of the diminished pressor responses. Isolated vessels gave lesser responses to drugs when cooled [12]. Also, vascular responses are decreased in respiratory acidosis [13], and hypothermic dogs are in respiratory acidosis despite mechanical hyperventilation [14]. The diminished blood volume [15] and the augmented viscosity of blood [16] observed in hypothermia, may also interfere with the response.

The onset of the pressor action was delayed during hypothermia, probably because of the increase in circulation time [6].

The extended duration of the pressor effect may be due to a slower inactivation of the drugs in the hypothermic state. Hypothermia can interfere in metabolic pathways of inactivation, as cooling depresses enzymatic activity in accordance to Van't Hoff rule. This hypothermic depression is known for O_2 consumption [19-23], renal function [24-25], nerve transmission [26] and extraction of bromsulphophtalein by the liver [27]. When animals are rewarmed, the duration of the pressor action returns to pre-hypothermic values, but the height of the response is still lower, indicating some failure for full respon-

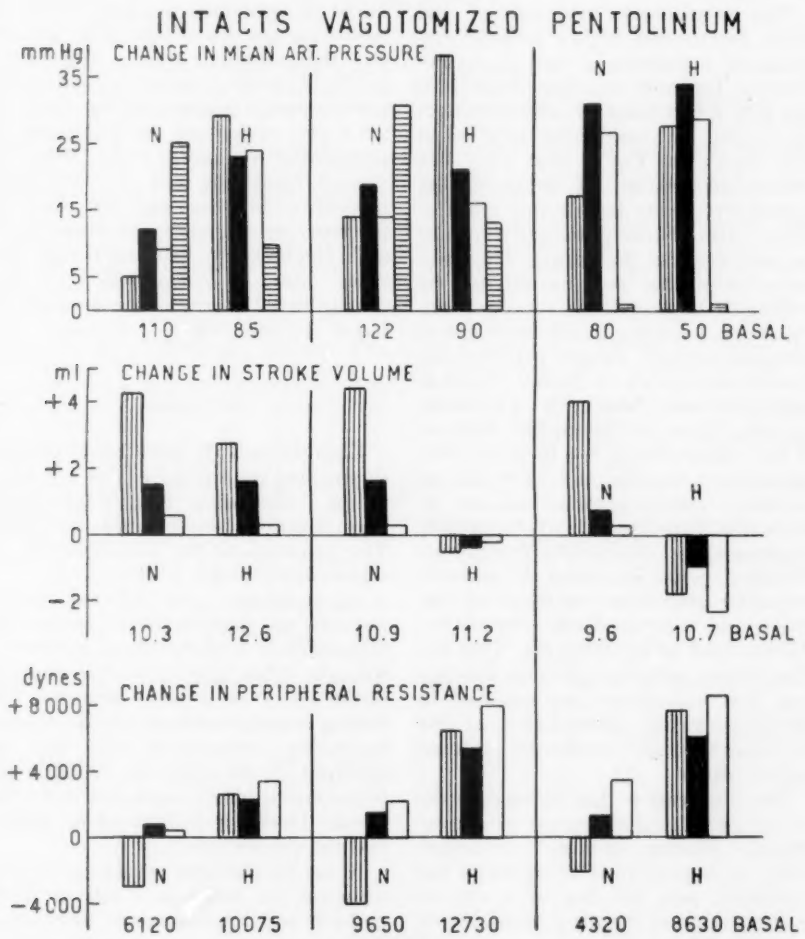

FIG. 4. — *Effects of the sustained injections of the drugs, in normothermia (N) and hypothermia (H).*

adrenaline = |||| *vertical lines*
noradrenaline = ■ *full column*
angiotensin □ *clear column*

carotid occlusion = ≡ *horizontal lines.*

At the bottom of the columns: mean value of the variable before injections.

ses, which is not dependent on temperature [29].

The continuous injection of the drugs determined higher pressure responses in hypothermia than in normothermia, but only in intact dogs. This fact may result from the additive effect of priming and continuous infusion as was shown in Fig. 3 for NA. The slower inactivation of drugs during hypothermia may favour this additive effect. Also, baroceptors activity may be involved in the results obtained, since the pressor response elicited by a drug is higher if the receptors are less active. Evidence that presoreceptors have less activity during hypothermia have been reported by Nashat [30], Malmejac [31] and Albers [32]. The same meaning have the significant decrease in the post-occlusion rise in blood pressure during hypothermia, after carotid occlusion test. Supression of the influence of baroceptors, either partially (vagotomy) or completely (ganglionic blocking agents) increased the pressure responses after the infusion of the drugs, and this was similar in normothermia and in hypothermia. This finding agrees with the previous assumption that baroceptors are involved in the hypothermic potentiation of the responses to the constantly infused pressor drugs.

The observation that adrenaline decreases peripheral resistance in normothermia (during sustained infusions) while in hypothermia it increases the resistance, may be due to a rise in concentration of the drug in the blood, because of delayed inactivation.

The marked hypotensive action of pentolinium tartrate during hypothermia suggests that if hypotension induced by ganglion blocking agents is used as a test for sympathetic activity [35], the vasomotor tone is augmented at 25° C. Evoked medullary potentials are increased during cooling down to 25° C [34], suggesting the possibility of augmented vasomotor tone. This increased vasomotor tone can be a component in the increased TPR observed in hypothermia, although Bullard [36] and Hegnauer [6] suggested that the main cause of augmented resistance during hypothermia is the increase in blood viscosity.

Summary

Hypothermia is associated with a diminished pressor activity of nor-adrenaline, adrenaline and angiotensin, when tested in single rapid injections. The duration of the pressor action is significantly longer at 25° C.

When drugs are injected continuously, the pressor effect obtained in hypothermia is higher than in normothermia. This may be due to the additive effect of priming and the following continuous dose. Blood pressure regulating mechanisms may also be involved in the observed hypothermic potentiation, since vagotomy and ganglionic blockade diminished or abolished the pressor effect.

If the hypotensive responses to pentolinium are taken as a test for sympathetic activity, vasomotor tone seems to be normal or augmented during hypothermia.

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PROCEEDINGS OF THE ARGENTINE SOCIETY OF BIOLOGY

(BUENOS AIRES, ARGENTINA)

September 1st, 1960

Submaxillary gland in the male rat under different hormonal treatments. J. J. ARGONZ AND J. M. DE CORRAL SALETA. *(Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires).*

Submaxillary glands of normal male rats treated with several hormones and hormonal combinations were studied. It was shown that testosterone was the most efficient hormone to increase the weight of the gland. Microscopically very abundant tubuli, full of intracellular granulations, were seen. Then came testosterone combinations with other hormones (STH, thyroxine, hydrocortisone). These last ones, when injected isolatedly, had no action at all. The results are discussed.

Physiological factors modifying the polyarthropathy produced by injection of mycobacteriae. R. H. HOUSSAY. *(Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires).*

When new born rats were injected with a suspension of mycobacteriae only a small proportion developed an arthritis. The incidence and the intensity increased when the rats were mature at the time they were injected. The greater incidence and the maximum of severity were seen in rats between one and three months old.

This variation was not due to the presence of the gonads. There was not an appreciable difference regarding to sex and no change was seen in gonadectomized animals.

Pregnancy occurring at the beginning of the incubation period resulted in a delay in the appearance of the arthropathy, or decreased its severity.

When it occurred at the end of the latent period, or during the acute phase, improvements of variable degrees and even disappearance of the lesions were seen. In chronic cases, improvement was less marked. After delivery an increase of symptoms was often seen.

Lactation did not modify the evolution of the process.

Action of ACTH and cortisone on Fe (ferritine) fixation in the normal liver of the rat. M. ROYER AND B. NOIR. *(Instituto de Gastroenterología, Institutos Nacionales de Salud, Ramos Mejía, Buenos Aires, Argentina).*

The injection of Fe^{59} (chloride or citrate) in rats through the peritoneum produces a quick formation of ferritine in the liver.

There is a constant and transient decrease of ferritine (and of Fe^{59} in hemoglobin) on the 7th day.

From the 25th until the 63rd day, the proportion of ferritine remains practically unchanged.

The action of ACTH and Cortisone is not very marked.

Effects of acute hypoxia on the erythroblastic proliferation of normal and nephrectomized rats. J. L. SCARO.

(Instituto de Biología de la Altura, Univ. Nac. de Tucumán, San Salvador de Jujuy, Argentina).

Using the statmocinetic test, the rate of erythroblastic proliferation in normal, nephrectomized and ureter-ligated rats, was studied.

Removal of both kidneys was followed by a highly significant decrease of erythroblastic proliferation. Erythropoiesis was essentially normal in rats with bilateral ureter ligation.

Nephrectomized rats when submitted to hypoxia, didn't show the highly significant increase observed in normal or ureter-ligated animals.

A proliferative response was observed after injection of erythropoietic factor into nephrectomized rats.

It may be concluded that the kidney plays a principal role in controlling the erythroblastic proliferation of the bone marrow in rats.

Reaction of yeast DPN-aldehyde dehydrogenase with analogues of diphospho-piridine-adenine dinucleotide (DPN). M. N. SCHWARCZ AND A. O. M. STOPPANI. *(Instituto de Química Biológica, Facultad de Ciencias Médicas, Buenos Aires).*

Baker's yeast DPN specific aldehyde dehydrogenase binds DPN analogues. The value of the apparent dissociation constant of the respective compounds are smaller (deamino-DPN, pyridine-3-aldehydeDPN, acetyl-3-pyridine-deaminoDPN and TPN) or higher (pyridine-3-aldehyde deaminoDPN, acetyl-3-pyridineDPN) than the constant of DPN. The adenine nucleotide is a competitive inhibitor of DPN whereas the pyridine nucleotide is not apparently bound by the protein. Adenine and nicotinamide are also competitive inhibitors with regard to DPN. DPN should link the protein through the adenine, nicotinamide and pyrophosphate groups. The carboxamide group is important for the reduction of DPN as the acetyl or aldehyde analogues are far less, or not active as hydrogen acceptors. The amino group of adenine seems less important than the carboxamide for DPN reduction, because acetyl-3-pyridine deaminoDPN is more effective than the respective amino compound and deaminoDPN is only about half active as DPN. The structure of the coenzime affects the formation and decomposition of the acetaldehyde-enzime compound, in agreement with the hypothesis of a ternary (acetaldehyde-enzyme-DPN) active compound.

Influence of changes of temperature on the action of oxygen on the glomerular filtrate of the toad. J. URANGA. *(Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires).*

Glomerular filtration was measured in toads *Bufo arenarum* Hensel at different temperatures, during the months of February, March, April and May, as well as the influence of hypophysectomy, lesion of the preoptic-hypophyseal tract and injection of 50 mU/100 g of oxytocin.

1) An increase in temperature induced a proportional increase in glomerular filtration.

2) At the same temperature glomerular filtration was higher in summer than in winter.

3) Hypophysectomy and lesion of the preoptic-hypophyseal tract induce an increase in glomerular filtration.

4) Oxytocin also increased glomerular filtration, but the sensitivity to this hormone varied according to the temperature.

(CÓRDOBA, ARGENTINA)
June 23rd, 1960

An attempt to detect chorionic gonadotrophins in some mammals. J. J. ASTRADA. *(Instituto de Investigaciones Médicas "Mercedes y Martín Ferreyra", Casilla de Correo 389, Córdoba).*

The present work was carried out to show whether there was any chorionic gonadotrophin in pregnant mice, rats, vizcachas (Lagostomus trycodactilus) and cows.

Urine, feces, blood serum, liver, placenta, uterus and fetus, the last only in mice and rats were studied. Amniotic fluid and milk were also studied in the pregnant cow. The biological test employed was the spermiation reaction in the toad.

It was not possible to show the existence of chorionic gonadotrophin in any case.

Study of the effect of enzymes and pH on human and amphibian gona-

dotrophins. J. J. Astrada and L. S. de Caligaris. *(Instituto de Investigación Médica "Mercedes y Martín Ferreyra", Casilla de Correo 389, Córdoba).*

The action of ptyalin, pepsin and trypsin on the hypophyseal gonadotrophins of the toad and on human chorionic and hypophyseal gonadotrophins was investigated.

The effect of pH on the same gonadotrophins was also studied.

Spermiation and mouse uterine response were the biological test employed.

Ptyaline inactivated neither the hypohyseal gonadotrophins of the toad nor human chorionic gonadotrophins. They both were inactivated by trypsin.

Human hypophyseal gonadotrophins were inactivated by ptyalin and trypsin.

It was not possible to show the effect of pepsin.

The hypophyseal gonadotrophins of the toad were the most sensitive to pH changes.

Action of pressure on circulatory flow and urine in the perfused kidney of the toad. F. A. Madoery de Bonet. *(Instituto de Fisiología "Prof. O. Orías", Facultad de Ciencias Médicas, Univ. de Córdoba, Santa Rosa 1085, Córdoba).*

Perfusions were performed in 85 isolated kidneys of *Bufo arenarum* Hensel, weighing from 48 to 160 g, almost all of them males. Room temperature ranged from 25 to 30° C. Perfusions were performed during 3 hours: 1) by artery and vein simultaneously; 2) by artery, or 3) by vein. With increasing pressures, there was a parallel rise of flow volume and urine formation. Lowering the pressure, both volumes decrease. In the venous perfusion the flow was much more constant. There is a greater parallelism between variations of perfusion volume and urine when perfusion is made only through the artery than when both vessels are perfused together, because renal-portal vein contributes to the renal flow and it has no influence on the urine formation.

Perfusion of isolated kidney in "Bufo paracnemis". Florentina A. Madoery de Bonet. *(Instituto de Fisiología "Prof. O. Orías", Facultad de Ciencias Médicas, Santa Rosa 1085, Córdoba).*

Sixteen perfusions were performed in isolated kidneys of *Bufo paracnemis*, of both sexes, weighing 261 to 860 g between autumn-winter months.

The line which correlates volumes perfused against urine formation, in the first hour of observation has the following value: $y = 0.065\ X - 0.23$.

The behaviour of these amphibia is similar to *Bufo arenarum* Hensel.

Increasing or decreasing the flow volume causes parallel variations in urine formation.

Effect of insulin in the perfusion of the isolated liver of "Bufo arenarum Hensel". I. Flores. *(Instituto de Fisiología "Prof. O. Orías", Facultad de Ciencias Médicas, Santa Rosa 1085, Córdoba).*

Fifty three isolated livers of *Bufo arenarum* Hensel were perfused with commercial insulin "Lilly", slow insulin "Novo" and "Lilly" free from hyperglycemic factor, in Ringer concentrations varying from 2 to 20 U per 1000 ml.

The spontaneous liberation of glucose increases notably through the action of the commercial insulin "Lilly" and somewhat less on adding slow insulin "Novo". The effect can be observed after 15 minutes, reaches its maximum before the hour and persists without great variations.

The Lilly insulin, free from hyperglycemic factor reduces the liberation of glucose since its administration, the effect increasing with the passing of time. Ten units practically inhibit glucose liberation.

(Buenos Aires, Argentina)
August 4th, 1960)

Recovery by hormones of the alterations produced by castration in the submaxillary gland. J. J. Argonz. *(Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires).*

Submaxillary glands of castrated rats, controls and injected with various hormones, were

studied, and the results were compared with those of normal rats. It was proved that castration produced a decrease in the diameter of tubuli and number of tubuli by microscopic field. Testosterone was the only hormone capable of restoring the tubuli. The weight of the gland was not affected by castration, but was increased by testosterone.

Changes produced by glucagon in the islets of Langerhans of the snake "Xenodon merremii". A. F. CARDEZA. *(Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires).*

Microscopic studies of the pancreas, liver and kidneys of the *Xenodon merremii* snake, daily injected with glucagon, were performed.

At 24 hours, hyperglycemia, decrease in the number of alpha cells and partial degranulation of beta cells was seen. These alterations progress up to 6 days. At 30 days the hyperglycemia was only moderate, but marked decrease in the number of alpha cells, degranulation and vacuolization of beta cells with moderate glycogenic infiltration and some mitosis, were observed. Liver and kidney showed progressive sequential fatty changes.

Decrease in the number of alpha cells suggests the possibility of glucagon secretion by this type of cells. Alterations of beta cells could be interpreted as a result of the hyperglycemic stimulation of these cells.

Hemodynamics in the shock due to occlusion of the porta vein. M. J. GUERRERO, R. H. MEJÍA, O. RETTORI, M. E. DESCALZI, E. STRAJMAN AND M. BRAUN. *(Instituto de Fisiología y Cátedra de Física Biológica, Facultad de Ciencias Médicas, Univ. de Buenos Aires).*

1) In 32 dogs submitted to hypovolemic shock by acute ligation of the portal vein, mean blood pressure, cardiac index, circulation time and total peripheral resistence were studied.

2) A marked decrease of cardiac index and mean blood pressure, together with a rise in total peripheral resistance and lengthening of the circulation time was seen.

(BUENOS AIRES, ARGENTINA)
July 7th, 1960

Recovery of the submaxillary gland by hormone treatment in the hypophysectomized rat. J. J. ARGONZ AND J. M. DE CORRAL SALETA. *(Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires).*

Submaxillary glands of hypophysectomized or normal rats were studied. It was verified that hypophysectomy brought a diminution of weight of the gland and a reduction of the diameter of acini and tubuli. It was tried to restore the normal state by the administration of various isolated hormones or of hormones in combination. The most active combination was testosterone and thyroxine.

Experimental polyarthropathy produced by injection of dead mycobacteriae. R. H. HOUSSAY AND A. F. CARDEZA. *(Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires).*

Arthropathy in rats injected intradermically with mycobacteria in oil emulsion was studied.

An inflammatory reaction was observed in the place of inoculation, the presence of which seemed to influence, but was not necessary for the appearance of the arthropathy.

The course could be acute or become chronic. It could involve one, many or almost all the peripheral joints and the spine, specially the tail.

The aspect of the lesions during the acute and chronic stages of the process is described.

It is a polyarthropathy of inflammatory type during the acute phase, infiltration of mononuclear cells and proliferation of sinovial cells and periosteum predominate during the subacute stage while fibrosis and ossification are prominent in the chronic period.

Fibrinoid material and perivascular lesions of infiltrative type in the visceral interstitium (especially heart and lung) were seen.

X-ray examination showed alterations in the articular light and bone structure; these alterations were very marked in the old lesions.

The nature of the arthropathy and the reasons leading to a possible immunologic mechanism are discussed.

Liver, mitochondria and fatty changes in early choline deficiency. Quantitative electronmicroscopy and histochemistry. E. A. PORTA, W. S. HARTROFT AND J. S. MEYER. *(Department of Pathology, Washington University, St. Louis, Mo., USA).*

Changes produced in livers of rats by 4 days of choline deficiency are adaptable to quantitative electron-microscopic and histochemical studies. Wistar, Albino, male rats were fed a choline deficient diet supplemented with 0.5 % choline chloride of a standard commercial ration. All rats were killed after 4 days and centrolobular hepatic cells studied by light and electron-microscopy. Surface areas of a certain number of cells in each animal were determined and numbers of their visible mitochondria counted. Rations of oblong to round mitochondria were established. Statistical analyses of results indicate that at 4 days the average number of mitochondria is the same for the three groups. However variations in the form of mitochondria became significant in the choline-deficient rats. The supposed mitochondrial swelling, if really exists, has not been detected by methods and conditions of this particular experiment. The significant difference of visible fat, as evaluated histochemically, between the deficient and the supplemented group was related with the action of dietary choline, but the variation of mitochondrial form could not be attributed to choline deficiency with the semisynthetic diet employed in this experiment.

Maintenance by corticoids of lactation in adrenalectomized rats. L. R. DE RUBINSTEIN. *(Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires).*

1) Adrenalectomy performed on the 4th day post-partum produced a non-complete but significant inhibition on lactation.

2) Lactation was partially maintained with daily administration of hydrocortisone and triamcinolone. Dexamethasone showed to be very active with the three dosis used, and occasionnally a complete replacement was obtained. With this drug a linear relation between the logarithm of the daily dosis and responses was observed.

3) Amphenone produced marked inhibition on lactation; the values were similar to adrenalectomized rats without treatment.

4) Histological differences were found between normal and operated rats without treatment, but no differences were seen between operated animals with treatment and controls.

Action of adrenalectomy on the pepsinogen in gastric mucosa of rat (Biochemical-Histological correlation). M. SCHRAIER, L. BIEMPICA, B. LOZZIO, P. MAZURE AND S. GORODISCH. *(Instituto de Gastroenterología, Institutos Nacionales de Salud, Ramos Mejía, Buenos Aires).*

The effect of adrenalectomy on the concentration of pepsinogen of the gastric mucosa of the rat was studied. The histological changes and their relations to the biochemical findings were discussed.

Adrenalectomy on rats produces marked decrease of pepsinogen of the gastric mucosa and the latter can be histologically related with the involution of zymogenic cells.

Irreversible inactivation of yeast DPN-aldehyde dehydrogenase by 1,10 phenanthroline. A. O. M. STOPPANI AND M. N. SCHWARCZ. *(Instituto de Química Biológica, Facultad de Ciencias Médicas, Universidad de Buenos Aires).*

Baker's yeast potassium-activated aldehyde dehydrogenase is irreversibly inactivated by 1,10-phenanthroline (OP) and to a lesser extent, by other metal-binding agents (8-hydroxyquinoline, α, α'-dipyridil, diethyldithiocarbamate and sodium azide). The action of OP increases with the purity of the enzyme preparation and the temperature. Metal-binding agents which produce enzyme activation, like cysteine, EDTA, diethyldithiocarbamate, 8-hydroxyquinoline, glycine and histidine, prevent inactivation with OP and with cysteine there is a nearly quantitative relationship between the activation and protection effects. DPN, and its analogues (TPN, APyDPN and DeamDPN) increase the action of OP, whereas pyrophosphate and its analogues (PyAIDPN and PyAldeamDPN) prevent it. DPNH and APydeamDPN do not affect the irreversible action of OP.

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micrón	μ	μ	kilogramo	kg	kg
milimicrón	$m\mu$	$m\mu$	gramo	g	gm
Angström	Å	Å	miligramo	mg	mg
microgramo	μg	μg	miliequivalente	mEq	mEq
hora	h	hr	Curie	c	c
minuto	m	Min	Millicurie	mc	mc
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